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BIOLOGICAL BULLETIN

"REVERSAL OF INHIBITION" BY ATROPINE, IN CATERPILLARS.

W. J. CROZIER,

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With a variety of invertebrate animals it has been found that strychnine exerts upon the neuromuscular structures an effect essentially similar to that which it displays in connection with the synapse-substance of vertebrate central nervous organs—namely, a decreased resistance to transmission, resulting in enhanced reactiveness; and a selective excitation of extensor elements, leading to opisthotonic curvature and to "reversal of inhibition" in responses involving reciprocal innervation. Representatives of the following groups are known to show behavior of this type under strychninization, and must therefore be accorded as a common property the possession of strychnine-receptive complexes suitably situated in their nervous organs: Platyhelminthia (*Planaria,*[1] *Bdelloura*[2]); Mollusca (*Chætopleura,*[3] *Chromodoris,*[4] *Limax,*[1] *Ouchidium,*[5] *Loligo*[6]); Echinodermata (*Thyone,*[7] *Asterias*[8]); Annelida (earthworm,[9] leeches[1]).

[1] Personal observation.

[2] Moore, A. R., *Jour. Gen. Physiol.*, I., p. 97, 1918.

[3] Crozier, W. J., *Jour. Gen. Physiol.*, II, p. 627, 1921.

[4] Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXIX., p. 261, 1919.

[5] Crozier, W. J., and Arey, L. B., *Jour. Exp. Zoöl.*, Vol. XXXII., p. 443, 1921.

[6] Moore, A. R., *Jour. Gen. Physiol.*, Vol. I., p. 505, 1919.

[7] Pearse, A. S., BIOL. BULL., Vol. XVIII., p. 42.

[8] Moore, A. R., *Jour. Gen. Physiol.*, Vol. II., p. 201, 1920; *Jour. Pharm. Exp. Therap.*, Vol. IX., p. 167, 1916.

[9] Knowlton, F. P., and Moore, A. R., *Amer. Jour. Physiol.*, Vol. XLIV., p. 490.

Since strychnine has been relatively unique among neurophil substances in producing effects of the kind specified, it is desirable to call attention to the fact that among insects (caterpillars) phenomena identical in their nature with the reversal of inhibition typically induced by strychnine may be produced through the action of atropine; whereas, with these animals, strychnine is singularly ineffective and in fact fails to produce "reversal of inhibition." This point is important for the conception of progressive chemical differentiation of the nervous system in the animal series. Moreover, it raises certain difficulties in the way of employing strychninization as a test for the presence of synaptic structures or their homologues;[10] but further study is required to permit discussion of this aspect of the matter.

The experiments had in view the general impression that insects (and other arthropods) are peculiarly resistant to strychninization.[11] A common method of testing the action of a drug has been to submerge the organism in an appropriate solution, or to introduce the material into the alimentary canal. The cuticula of arthropods, however, may seriously interfere with such trials; its permeability is but little understood. Similarly, with *Ascaris* Schroeder[12] reported that immersion for three hours in 0.5 per cent. solution of strychnine was without effect; but some time ago I observed that characteristic squirming movements could be induced by injecting weak strychnine solutions into the interior of the body.

Resort was accordingly had to hypodermic injection. With small insects such procedure is impracticable; moreover, it was desirable to use forms in which the nervous system had not undergone great condensation. Suitable material was found in certain large caterpillars, of which five species were employed: *Protoparce celeus, Samia cynthia, S. promethia, Automeris io,* and *Ceratomia catalapæ*. The last named was not quite so well adapted to the injection as were the first four, upon which most of the observations were made. Half-cubic-centimeter volumes of solution

[10] Parker, G. H., "The Elementary Nervous System," Philadelphia, 229 pp. 1920.

[11] Biberfield, J., *Ergebn. Physiol.*, Jahrg. 17, p. 175; and the fact is well known to economic entomologists.

[12] Schroeder, W., *Arch. Exp. Path. u. Pharm.*, Bd. XIX., 290, 1885.

could be injected without material loss of fluid through the puncture; sealing the puncture with celloidin, after withdrawing the injection needle, was found unnecessary, and indeed undesirable, as leading to local irritation. Strychnine injection was usually made laterally at about the level of the first prolegs; the needle, pointing anteriorly, was thrust through the cuticule of an intersegmental constriction. It may be stated at once that the level of the injection appeared not to influence the outcome.

Upon the injection of strychnine sulphate in concentrations less than 1:100 no efforts could be detected, beyond a momentary loss of the ability to creep; this was not specific and could be induced by injecting distilled water or Ringer solution. *Protoparce celeus* and other forms were observed to drink as much as 0.8 cubic centimeter or more of 1 per cent. strychnine sulphate.

In *Automeris io* injection of 0.5 c.c. of 1 per cent. strychnine sulphate led to twitching contractions of the abdominal musculature, sometimes symmetrical, sometimes better developed at one side; the legs and prolegs were quiescent, nonreactive to stimulation, and the prolegs particularly were pressed together. After 4 minutes a pinched proleg retracted its terminal comb, but no further response was observed; the body musculature was very "flabby." At about 10 minutes subsequent to injection there appeared a phenomenon, only partially reproduced with the other species, which is the most closely related among those observed to the "typical" strychnine effect. With the animal on its side, the body may be bent somewhat, either dorsally or ventrally. If the caterpillar be given a slight ventral flexure, rhythmic twitchings appear in the dorsal thoracic zone, which serve to make the body curvature distinctly concave dorsally; whereas, with a dorsal curvature initially impressed, the caterpillar remains in that position, although the dorsal twitchings persist. The twitchings of the dorsal muscle bands likewise continue when the larva is picked up by its posterior spine.

The stimulation of the contraction of dorsal muscle bands is similar to the stimulation of dorsal extensor muscles in other groups, but further evidence of characteristic strychnine action is lacking. It is probably significant that *Automeris io* is distinctly the most reactive, most "irritable," of the caterpillars studied.

With the other species employed in the experiments this effect was not seen. In each form used, however, strychnine in saturated solution (volume 0.5 c.c.) produced temporary but violent convulsive movements, incoördinated trembling movements of legs and prolegs, followed by quiescence, and often a rather strong *ventral* flexure and paralysis of the appendages. Twelve hours was sufficient to permit full recovery from the effects of even such doses of strychnine. Experiments with various aquatic larvæ (especially of *Psephenus*), and upon crayfish, further point to the very slight toxic effect of strychnine, and likewise show the detectable tendency to produce opisthotonic curvature. Crayfish, even when holes have been punctured in the cuticula, presumably facilitating absorption of the drug, were found to live for 9 days in a strychnine solution originally 1 : 1000 and slowly evaporated during the nine days that the test continued. The backward swimming of the crayfish is impeded by the strychnine, while there is maintained an incessant forward creeping.

In contrast with this behavior of the strychninized arthropod neuromuscular system, in annelids we observe typical "reversal of inhibition," although in the earthworm[9] it is perhaps more difficult to demonstrate than is true with leeches. Several species of leeches have been studied in dilute strychnine sulphate, and for present purposes the following findings may be cited: there is pronounced contraction of the dorsal longitudinal musculature, relaxation of the ventral, while in response to local irritation the customary behavior of longitudinal, transversal, and dorsoventral muscles is completely reversed by the drug.

Atropine.

Injection of 1 per cent. atropine sulphate, 0.5 c.c., produced in all forms studied an abrupt loss of creeping ability, within 1 minute of the injection; this was followed by a period of segmental tremblings, involving some ventralward contraction (the animals lying on the side), and within 5 minutes after injection there appeared a very striking reversal of the behavior of the prolegs. Normally, and especially, in their natural use, when the creature creeps on the edge of a leaf, slight tactile stimulation of the ventrum between the legs suffices to induce the extension of

the terminal combs of the prolegs, simultaneous extension of the proleg pair, and their apposition—the whole movement giving an effective embrace of a leaf margin. The muscles governing the extension of the terminal combs, those having to do with the movements of the prolegs themselves, and the muscles of the body wall,[13] all are involved in this coördinated movement of response to stimulation of the ventral body wall; and under atropine the movement is entirely reversed—combs are retracted, prolegs greatly retracted, body wall itself retracted rather than, as normally, protruded in the region between the proleg. This "reversal" was found only in connection with the prolegs, never with the thoracic legs.

Normally, lightly touching a proleg comb, or the skin between the prolegs, leads to extension of both members of the pair; then a subsequent light touch leads to the usual clasping motion; under atropine, touching comb or skin between prolegs results in the spreading apart and deep retraction of the prolegs. The reversal of the proleg response persisted for 20 hours or more.

Atropine is very effective in producing heightened irritability; and usually there was evidence, also, of a tendency to dorsal contracture—slight suggestion of an opisthotonic state (perhaps related to the lifting of the head when the normal animal is disturbed on a leaf).

In one or two cases transitory evidence of proleg reversal was obtained during observation of larvæ injected with nicotine solutions, and particularly with pilocarpine; it could not be produced at will with these substances, however, nor was trace of the phenomenon detected under treatment with a variety of other neurophil drugs. It is a characteristic and pronounced effect peculiar, in my experience, to atropine.

With strychnine, and less clearly with atropine, suggestion was had of the special stimulation of the dorsal muscles ("opisthotonus"). This effect is not specific, or at least is not so specific as the proleg reversal. Tetraethylammonium chloride ($m/64$) invariably led to striking opisthotonic spasms, due chiefly to contraction of dorsal muscles at the thoracic level. Chloroform (one third saturated) behaved somewhat in the same way, but more

[13] Cf. Peterson, A., *Ann. Ent. Soc. Amer.* Vol. V., p. 246, 1912.

vaguely. Ventralward contraction, especially at the anterior end, was always produced by nicotine, camphor, phenol, resorcin, and caffeine.

Chemical Differentiation.

It is of interest to make note of certain facts bearing upon the question of phylogenetic neuromuscular differentiation, from the standpoint of reactivity to drugs.

The larvæ used agree in showing that the following instances exert a very definite neuromuscular excitation—evidenced by spontaneous writhings, often of definite pattern, or by temporarily heightened responsiveness, or both:

* nicotine ..... (1:500)
* picrotoxin ..... (1:240)
* camphor ..... (saturated)
* pilocarpine ..... (1:150)
* adrenaline ..... (1:1000)
phenol ..... (1:2000)
* resorcin ..... (1:1000)
caffeine ..... (saturated solution; 1:200, without effect)
* tetraethylammonium hydroxide (*m*/64)
chloroform ..... (1/3 saturated)
* atropine ..... (1:500)
strychnine ..... (1:100)

* (Especially exciting.)

Creatine, *m*/8, gave only slight temporary paralysis, probably not specific.

These facts suggest obvious differences when the behavior of the caterpillars is compared with that of other invertebrates. The action of atropine, and the relative lack of effectiveness of strychnine, point to parallelisms with conditions in cœlenterates,[14] rather than with forms closer akin. Crayfish injected with strychnine fail to yield evidence of "reversal" in the use of muscle groups, and atropine has a marked effect in stimulating the thoracic ganglia. But in how far the peculiarities here revealed are arthropodan characteristics cannot as yet be said.

In comparing a series of larvæ, such as the *Samias* and *Io*, one is struck by the fact that the *Io* caterpillar, provided with urticant

[14] Moore, A. R., *Proc. Nat. Acad. Sci.*, Vol. III., p. 598, 1917.

spines, shows normally a rather violent, jerky response to even mild stimulation. This is obviously a point of some significance, ethologically, since it increases the effectiveness of the "hairs" in penetrating and stinging. But under appropriate drug injection the quick, "snappy" type of behavior rather peculiar to *Io* can be easily brought about, for example, in a *Samia* caterpillar. Pilocarpine gave especially instructive comparisons of this sort. The sensory thresholds are so lowered by this substance that a *Protoparce* or *Samia* twists sharply in response to a single light touch, contracting at the place of stimulation, just as *Io* normally does. Biting reactions are likewise enhanced, so that *Samia* snaps savagely at its own spines encountered in writhing, or at forceps touching the head, much as *Io* normally does. The production of behavior normal to one species in another form characteristically more sluggish shows that the drug produces no *new* forms of response, but merely accentuates types of reaction for which the structural pathways already exist. Some light is thus given on structural basis of behavior differences in related forms.

The action of atropine shows clearly that reciprocal innervation exists in these insects, at least so far as concerns the action of the antagonistic muscle groups of the prolegs and walls of the segments. That strychnine, even in high concentration, fails to react in its characteristic manner with the synapses necessarily involved in these reciprocally acting nervous elements, shows that strychnine may fail to reveal the presence of synapses; and also that synapses even in types so closely related as annelids and arthropods may differ from one another;[15] or else that the "reversal" phenomena induced by atropine are brought about through action of the latter upon a locus distinct from that usually reacting with strychnine.

[15] Cf. Cushney, A. R., *Science*, N.S., Vol. XLIV., p. 482, 1916. It may be suggested that the slight acidity of caterpillar hæmolymph (p_H 6.8–6.6; Jameson and Atkins, *Biochem. Jour.*, Vol. XV., p. 209, 1921) might influence the action of the drugs; but I doubt that the low toxicity, at least, of strychnine can be explained in this way.

NOTES ON SOME PROBLEMS OF ADAPTATION: 9. CTENIDIAL VARIATION IN CHITON.[1]

L. H. SNYDER AND W. J. CROZIER,
(1901–) (1892–)

ZOÖLOGICAL LABORATORY, RUTGERS COLLEGE.

1. The respiratory organs of the Amphineura comprise a more or less extensive row of branchial filaments on either side of the body, in the groove between girdle and foot. Within the species it is known that the number of these ctenidia is variable. It is likewise known that the right and left ctenidial sets of an individual may differ in number of included gills. Published observations on these points have been summarized by Pelseneer (1920, p. 201). It appears that in some species the number of ctenidia may increase with the size of the animal; in others it seems to decrease; while in still others no correlation is very evident between size of animal and number of gills. We have sought to determine the nature and extent of ctenidial variation in *Chiton tuberculatus* of the Bermudas, having in mind the possibility of evidence upon the following points:

(1) Ctenidial variation as correlated with size (age),
(2) with sex, and
(3) with habitat; and
(4) indications of asymmetry.

The gill plumes were counted in 100 individuals, of a range of sizes. The length of the fourth shell-valve, along its mid-line, was measured as an index of size. By means of graphs such as that given by Arey and Crozier (1919, p. 164) the length of the fourth valve may be translated into terms of the length of the individual; the curled condition of many of the specimens, as preserved, precluded direct determination of their lengths. It is sufficient to note that the length of the shell-valve varies directly with the total size of the animal, though the relation is not exactly a linear one,

[1] Contributions from the Bermuda Biological Station for Research. No. 141.

owing to the effects of erosion and its consequences (Arey and Crozier, 1919).

The total number of ctenidia is at first 64–66, and increases in a fairly regular manner as growth proceeds. The average maximum number is about 98. The data are given in Table I.

TABLE I.

Total number of branchiæ	Length of valve 4 in mm. 0	1	2	3	4	5	6	7	8	9	10	11	12	13	
64-66	2														2
67-69															
70-72															
73-75	1	1													2
76-78		1	1												2
79-81		1	1	1											2
82-84			2	1	2	1									6
85-87		2				1						1			4
88-90				2	4		1	1	1						9
91-93			1	2	4	6	2	1			1		1	1	19
94-96			3		1	4	1	1	2	3		1			16
97-99						1	2	10	2		1		1		17
100-102						2	1	4	4			1	1		13
103-105								1	3						4
106-108							1	1				1			3
	3	5	8	6	11	15	8	19	12	3	2	4	3	1	100

DISTRIBUTION OF TOTAL NUMBERS OF CTENIDIA IN RELATION TO LENGTH OF FOURTH SHELL-VALVE; ALL INDIVIDUALS.

2. Since the number of gills is determined in part through growth, it is of interest to learn if the extent of the gill series is influenced by sex. Fig. 1 makes it plain that such influence, if real, must be slight. It is possible that there is on the average a slightly higher number of ctenidia in the males, when the comparison is made of males and females equal in size; on the basis of *age* this slight difference is increased, for the females are the larger, at the same age (unpublished data). It is doubtful if this

difference can be correlated with relative activity. New gills are in all probability added at the posterior end of the ctenidial series. Occasionally gill plumes are observed with divided tips (such as have been figured by Pelseneer, 1920, p. 204).

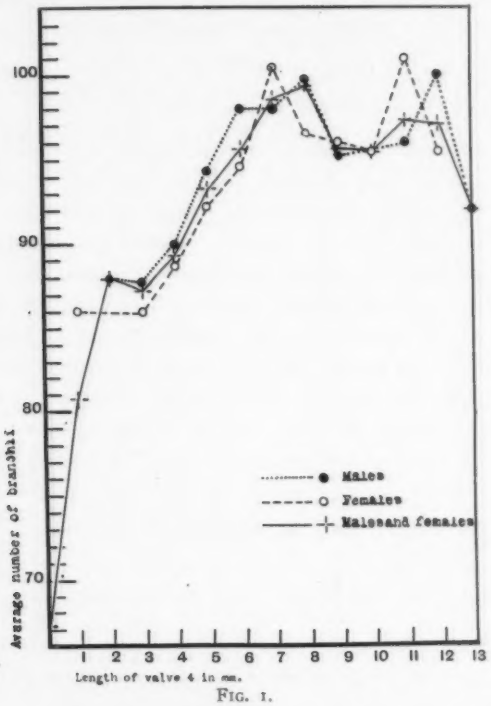

FIG. 1.

It appears from Fig. 1 that the full number of ctenidia is established before maximum growth is attained. It is unlikely that there is a real *decrease* in the number of ctenidia with extreme age (cf. Table I.). The number of gills is frequently mentioned as a subsidiary character in descriptions of species, hence recognition of the variation we record may be of taxonomic importance.

3. This inquiry began with the thought that the total number of gills might be influenced by habitat. The number of individuals at our disposal is small, but sufficient for preliminary study of the point.

Our *Chiton tuberculatus* were secured from two well-contrasted situations (cf. Crozier, 1918): an exposed ocean beach on the south shore of Bermuda (here referred to as "South Shore"), and the shores of an enclosed sound, Great Sound. In Fig. 2 the mean ctenidial frequency for each size class is contrasted for these two locations. Clearly there is no great difference between the

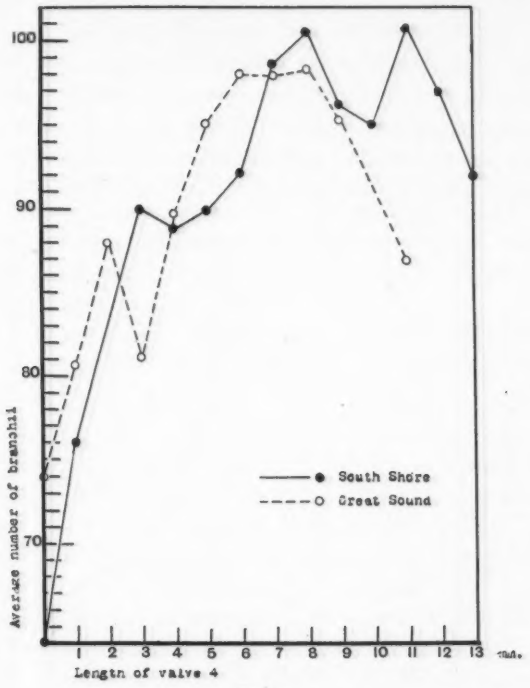

FIG. 2.

two sets of observations, though the lower number of ctenidia in the younger animals from the South Shore may perhaps prove significant.

4. Is it possible that the asymmetry of gasteropods is in some manner foreshadowed among the chitons? The two liver-lobes of young chitons are at first symmetrical, but later the right lobe becomes the smaller and is pushed anteriorly (Pelseneer, 1906,

p. 44); it has been noted that the number of the auriculo-ventricular connections of the heart may be dissimilar on the two sides (Pelseneer, 1897, p. 23); several instances of valve-fusion seemed asymmetrical (Crozier, 1919); and the existence of minor asymmetries in the two gill series has been recorded several times (Pelseneer, 1897; 1920, p. 201). But the published data fail to cover the possibility of definite correlation with superior growth on the right or on the left side.

Our counts show that the distribution of asymmetries of the ctenidia follows a purely random course; the absolute amount of asymmetry, moreover, is small. When excess of gill-plumes on the right side as compared with the left is reckoned as *plus*, on the left side as compared with the right as *minus*, a frequency curve is

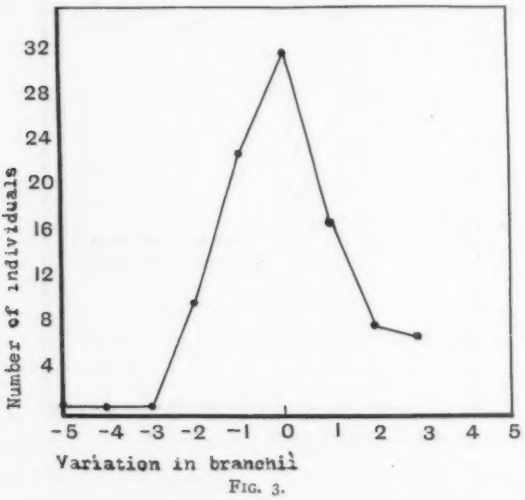

FIG. 3.

obtained (Fig. 3) indicating an essentially chance distribution. It follows that the asymmetry is merely an accident of growth, giving a measure of the independence of the two sides in forming new gills. On this view, the extent of ctenidial asymmetry should increase, as a rule, with size. Table II. shows that this is the case.

TABLE II.

RELATION OF ASYMMETERY (EXCESS NUMBER OF GILLS ON ONE SIDE) TO SIZE (CLASSES ACCORDING TO LENGTH OF VALVE 4.)

	Class Centers.			
	0.2 mm.	0.5 mm.	0.8 mm.	0.11 mm.
0.....	12	5	7	5
1.....	4	19	12	4
2.....	2	8	7	1
3.....	1		5	1
4.....			1	
5.....		1		

There is no apparent correlation of asymmetry with sex or with habitat. In the majority of cases the number of gill-plumes differs on the two sides, but the difference is usually not more than one. The asymmetry can therefore hardly be connected with the torsion of the body in gasteropods.[2]

Summary.—In *Chiton tuberculatus* at Bermuda the number of ctenidia increases from 32 on either side, in individuals about 1.0 cm. long, to an average of 49 on a side in individuals of the largest size (9–10 cm. long). The increase in the number of gills is at first quite rapid, so that the maximal number is achieved before the animal is 6.0 cm. long. The mean number of ctenidia is a little less in females than in males of the same size or age.

On an exposed ocean beach these chitons have the same number of gills as found in the population of an inclosed sound.

Minor asymmetries in number (1 to 5) are the rule when the two gill series of an individual are compared. Asymmetry follows a random distribution; it is an accident of growth and has no morphological significance.

CITATIONS.

Arey, L. B., and Crozier, W. J.

'19 The Sensory Responses of *Chiton*. Jour. Exp. Zoöl., Vol. 29, pp. 157–260.

[2] Two instances were found of a curious malformation, which may be noted here. At the level of the fourth valve a tough "bridge" of tissue had grown across from the lateral wall of the foot to the girdle, forming an arch over the ctenidial channel. These "bridges" were about 3 mm. wide.

Crozier, W. J.

'18 Growth of *Chiton tuberculatus* in Different Environments. Proc. Nat. Acad. Sci., Vol. 4, pp. 325–328.

'19 Coalescence of the Shell Plates in *Chiton*. Amer. Nat., Vol. 53, pp. 278–279.

Pelseneer, P.

'97 Sur la morphologie des branchies et des orifices rénaux et génitaux des chitons. Bull. Sci. Fr. et Belg., tom. 31, p. 23–30.

'06 Mollusca; in: Lankester's Treatise on Zoölogy, Pt. 5, 355 pp.

'20 Les variations et leur héréditié chez les Mollusques. Mém. Acad. Belg., Cl. Sci., sér. 2, tom. 5, 826 pp.

CHROMOSOME STUDIES ON THE DIPTERA. IV. INCOMPLETE SYNAPSIS OF CHROMOSOMES IN *DASYLLIS GROSSA* FABR.

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INTRODUCTION.

In the robber fly, *Dasyllis grossa,* synapsis of chromosomes in spermatogenesis (and possibly in oögenesis) appears to be partially inhibited, in such a manner that three of the pairs of chromosomes undergo synaptic association for only a portion of their length. The behavior is relatively uniform and constant in these particular chromosomes and indicates that different regions of a chromosome behave differently as regards synapsis. The present account deals primarily with this feature of chromosome behavior and with its possible bearing on cases of abnormal cross-over values in genetical experiments with *Drosophila.*

The description of spermatogenesis is taken largely from one specimen of *D. grossa* Fabr., kindly identified by Mr. C. W. Johnson; but it applies in a general way to all of the *Dasyllis* material I have studied (a dozen or more specimens, representing *D. grossa* Fabr., *D. thoracica* Fabr., and probably two or three more species). I am not certain that all of these agree in detail, but each one shows indications of incomplete synapsis in one or more pairs of chromosomes.

Dasyllis provides particularly favorable material for a study of chromosome behavior during spermatogenesis, because of the small number and relatively large size of the chromosomes; the large size of the nuclei; the relatively enormous number of cells present, representing all stages from early spermatogonia to spermatozoa; and particularly the serial orientation of successive stages throughout the tubular testes, making it easy to trace the chromosome behavior step by step through the growth period. In addition, it should be mentioned that there is no contraction or synizesis stage, and no true "diffuse" stage when the chromatin can not be seen.

In its general features spermatogenesis in *Dasyllis* resembles that in *Asilus sericeus,* as described by Metz and Nonidez ('21), and no attempt will be made to give a complete account here. The technique employed in the present work was the same as that described in the preceding paper. The material was fixed in strong Flemming and stained in Heidenhain's iron hæmatoxylin.

Dasyllis is unique among the Diptera thus far studied in possessing an unpaired X-chromosome in the male. This is true of all the material I have studied in the genus. The other Diptera have all been of the X-Y type. In an earlier paper (Metz, '16, p. 243) *Dasyllis* was tentatively considered to possess a Y-chromosome, but additional material has shown clearly that no Y is present unless it is so minute as to be practically invisible.

I am indebted to Miss M. S. Moses for assistance in the laborious work of examining the ovaries in connection with the observations on oögenesis, and to Miss Ruth Lincks for making the drawings for the accompanying figures.

Spermatogonia.

The spermatogonial chromosome group of *D. grossa* consists of three pairs of V-shaped and one pair of J-shaped (atelomitic) autosomes, and the unpaired X, as shown in Fig. 1. The J-shaped pair appears straight in this figure, but in later metaphases its subterminal spindle fiber attachment is evident. The V-shaped pairs are of different sizes, the smallest being readily distinguishable from the other two. Thus the X and two of the autosome pairs may be identified individually.

As in all of the other Diptera that I have studied, the paired association of chromosomes is persistent through successive spermatogonial generations, and into the final spermatogonial anaphase. In spermatogonial prophases (Figs. 2 and 3) the pairing of the large chromosomes does not seem to be as intimate medially as in most other flies—a fact which suggests that the peculiar repulsion or lack of attraction exhibited in the growth period is existent here also.

The Spermatocyte Growth Period.

In *Dasyllis* the spermatocyte nucleus is relatively large at the beginning of the growth period; and partly on this account, per-

haps, the growth period does not involve an increase of more than about 30–50 per cent. in nuclear diameter.

The description of the growth period may best be taken up at a point shortly after growth has begun (returning later to a consideration of the earliest stages). At this time the chromosomes, with the exception of the condensed X-chromosome, are in the form of long, deeply staining threads, closely applied to the nuclear membrane. There are four pairs of threads, in one of which (apparently the smallest) the two members are usually closely associated throughout their length—*i.e.*, the synaptic association is complete. In each of the other three the association is evident at each end but toward the middle the two components diverge to form large loops. Each pair of threads is very long, frequently extending more than half way around the circumference of the nucleus, hence it is practically impossible to represent them all in one figure. Individual pairs, however, are shown in Figs. 7 to 10. Most of these are complete, but in some (*e.g.*, Fig. 9) one or both ends of a pair may be cut off. To one of the three looped pairs (apparently the largest) is attached a large dense body (Fig. 7), which serves to identify this pair throughout the entire growth period. This body (see page 258 for description) is attached to both members of the pair in the looped region and normally lies at a point near one end of the loop—a position which it occupies with surprising regularity.

The three large pairs of chromosomes at this stage give the appearance of having undergone synapsis only near their ends—the threads having remained well apart medially for about one third to one half their length. In the smallest pair, as noted above, synapsis is usually complete, but in a few cases a small loop is present. The chromosome on the left in Fig. 9 appears to be such a case. Throughout the remainder (80 per cent. or more) of the growth period the condition of the chromosomes is maintained with relatively little change. The cells and nuclei grow somewhat and the chromosomes become more condensed and hence more easily examined. The X-chromosome remains condensed throughout. Successive stages are represented by thousands of cells and the transformation during growth is so gradual that scarcely any change is observable from one cyst to another down

the testis. It seems certain, therefore, that no important stage has been overlooked in this region.

Figs. 11 and 12 represent entire, or almost entire, nuclei showing the condition maintained by the chromosomes up to the end of the growth period. In each of the three large pairs of chromosomes the two components diverge medially in the form of a loop; while in the shorter pair they are usually closely applied throughout their length, with only occasionally a small loop visible. In Fig. 11 the entire contents of the nucleus are drawn in position; in Fig. 12 the pairs are transposed to facilitate examination, but are all taken from the same nucleus.

During the late prophase, as the chromosomes are about to go on the spindle, they become so condensed and shortened that in many cases the loops become closed (Fig. 13). The line of separation between the two chromosomes, however, is still evident, and it is practically certain that no intimate association occurs here.

It appears, then, that in the case of the three large pairs of chromosomes synapsis has been incomplete, unless it occurred at a very early stage in the growth period and was followed by a secondary opening out to form the loops. The evidence from the early stages may now be examined from this point of view.

The Early Growth Period.

The early growth stages resemble those of *Asilus sericeus* (Metz and Nonidez, '21) in a general way, but the details differ materially. In *Asilus* homologous chromosomes become closely associated in the final spermatogonial telophase and remain thus as they draw out into threads. Just before they draw out (stage *b*) the pairs look like single chromosomes relatively clear cut in outline. In *Dasyllis* the chromosomes are likewise paired; but the pairs form loose, irregularly granulated aggregates, giving little or no indication of the intimate association seen in *Asilus*.

The aggregates become more loose and irregular in structure as growth proceeds, and then draw out into irregular, granulated threads. Only two bodies remain condensed: one the X-chromosome and the other the "dense body" attached to the large pair of chromosomes. As the aggregates spin out into threads a more intimate association becomes possible. Unfortunately as the spin-

ning out proceeds the threads become entangled and lose much of their staining capacity, making this the most difficult stage to analyze. In fact, it is impossible to trace all of the chromosomes in any one nucleus. It is significant, however, that as soon as the aggregates have elongated two kinds of threads may be observed: single and double; and occasionally in favorable nuclei the two members of a double one may be seen to diverge in the form of a Y, or may even form a loop essentially like those of later stages. The most convincing evidence as to the nature of events at this time is obtained from an examination of the threads attached to the "dense body." As has been noted above, this body in later stages is attached to the two single threads making the loop in one of the large chromosome pairs. By following this structure through the early stages, then, it should be possible to determine whether or not the loop is present here also. A careful study of this feature has convinced me that the loop is normally present throughout the early stages—from the time the chromosomes first elongate. In some cases the entire structure may be seen, as shown in Figs. 4 and 6, and in others it is evident that the threads running out from the dense body are single, not double. The fact that single threads and occasionally loops (Fig. 5) may also be seen in other parts of the nucleus makes it seem almost certain that the same conclusion applies to the other large chromosomes. It is practically impossible to differentiate the smallest chromosome pair from the others in the early stages, hence I have been unable to determine whether or not it possesses a loop at this time. A little later the chromosome pairs move to the periphery of the nucleus and become separated sufficiently to permit of individual analysis—which brings them into the stage with which our description began (Figs. 7 to 9).

It is possible, of course, to assume that the chromosome pairs do not behave synchronously in the very early stages, and to imagine them undergoing, one at a time, a complete synapsis followed immediately by a partial opening out into loops. This would account for the constant presence of both single and double threads in the nucleus. Such an explanation seems improbable, however, from analogy with other forms, and especially in view of the evidence furnished by the chromosome pair attached to the

dense body, which may be traced through all the stages. The conclusion seems justified, therefore, that synapsis does not occur in the looped regions of the three large chromosome pairs.

The "Dense Body."[1]

This body, which appears to take the place of the nucleolus in other flies, has such a consistent connection with one of the large pairs of chromosomes throughout the growth period that its structure and history may be considered in some detail. It is usually ovoid or spherical, deeply staining in iron hæmatoxylin, and clear cut in outline. In some cases it is clearly bipartite in structure, and rarely it is divided into entirely separate parts. As already noted, it is attached to both members of one of the large chromosome pairs, probably the J-shaped pair. When it is divided into two parts, as in Figs. 17 to 19, this independent attachment is shown clearly. Associated with the dense body, on the side opposite the attachment to the chromosomes, is an achromatic structure (presumably a plasmosome), of variable size and irregular outline, as shown in Figs. 11, 12, and 18.

The origin of the dense body can not be traced accurately enough to demonstrate that it arises directly and equally from the two members of the one pair of chromosomes, but its subsequent history (attachment, bipartite structure, behavior in late prophase) suggests such an origin.

During the prophase of the first spermatocyte division, as the chromosomes go on the spindle, the dense body diminishes in size. It gives the appearance of being taken up, in part at least, by the attached chromosomes, for there is little indication of any of it diffusing or breaking off. It separates into two components at this time, coincident with the separation of the two chromosomes (Figs. 20, 21) preparatory to the reduction division. They persist in this condition up to, and perhaps through, the metaphase, as do the somewhat similar "chromosome vesicles" observed by Carothers ('13) in the Orthoptera *Brachystola* and *Arphia*. I have not attempted to follow their history beyond this point.

[1] Since the chemical nature of this body is not known it has been thought better to apply a descriptive term to it, rather than to call it a nucleolus, karyosome, or chromosome vesicle, each of which it resembles in certain respects.

The Maturation Divisions.

No special interest attaches to these and they will be passed over rapidly. The first division is reductional for autosomes and X-chromosome. The former appear as dyads with little evidence of a tetrad structure. Such a structure probably exists, however, and further extraction would perhaps bring it out, for in early anaphase each component of the dyad is itself clearly double. The X-chromosome goes to one pole in the first division and divides in the second. Metaphases of the first division and of the two types of second division are shown in Figs. 14 to 16.

Oögenesis.

Knowing the unusual behavior of the chromosomes during spermatogenesis, it would be of especial interest to determine whether or not similar phenomena occurred during oögenesis, but this has proved to be a very difficult task. Owing to the nature of oögenesis only a few, rather widely separated stages can be found in any one ovary, and the story has to be pieced together from an examination of many specimens. The orientation of stages, especially the early ones, is very difficult. Consequently I am only able to record a few observations at this time.

The chromosome group of the female, as expected, consists of five pairs—differing from that of the male only in the presence of two X-chromosomes instead of one (Fig. 22).

Near the apex of the ovarian tubules, in the region of transition from oögonia to oöcytes and nurse cells, are found nuclei with chromosomes such as those represented in Fig. 23 (probably oögonial) and in Figs. 24 to 28. In the latter very obvious loops are present, bearing a strong resemblance to those in the spermatocytes. These chromosomes are not pulling apart on the spindle, as their outlines might suggest, but are in resting nuclei, or at least nuclei that are not actively preparing to divide. Whether they represent the final generation of undifferentiated ovarian cells or represent an early growth stage of oöcytes and nurse cells, I am unable to determine. If the latter, then they may correspond to spermatocytes in the aggregated stage *b*. In any case, the condition seems to parallel that found in the male, for I have not

observed such loop structures in any other Diptera. This gives some ground for expecting similar synaptic phenomena in the two sexes in regard to the feature with which we are concerned here.

After the growth period is well under way and the oöcytes are clearly differentiated from the other cells, the chromatin appears in the form of large, irregular, heavily granulated aggregates whose structure I am unable to analyze. Before this, however, there appears to be a diffuse stage in which scarcely any chromatin is visible in the nucleus. My present material is not adequate for a detailed study of these stages and their treatment must be deferred.

Discussion.

The above facts, it is believed, provide strong support for the conclusion that, in the male *Dasyllis* at least, synapsis is not uniform throughout the length of the chromosomes, but may, in the case of certain chromosomes, occur only in the terminal regions, leaving the homologous members separated near the middle.

So far as I know, no equally clear cases of this sort have been described before, although figures that suggest a similar condition are given by Mohr ('16, Figs. 85–90) for *Locusta viridissima,* by Robertson ('16, Fig. 163) for *Chortippus curtipennis,*[2] and by Wenrich ('16, Figs. 73–78) for *Phrynotettix magnus.* These are interpreted by the authors as cases of delayed synapsis (*Locusta* and *Phrynotettix*), or early separation of threads after synapsis (*Chortippus*); but it is possible that with further study some of them may prove to be cases of incomplete synapsis. Professor McClung informs me that similar conditions may exist in other Orthoptera on which he has made preliminary observations.

It has also been suggested to me by McClung that the absence of synapsis in the median part of the long J- and V-shaped chromosomes in *Dasyllis* may be due to these chromosomes each being compounded of two rod-like chromosomes united end to end, as he has found chromosomes to be united in *Hesperotettix* (McClung, '17). This offers an attractive lead toward an explanation, but it is necessary to assume some other influence as well, else all compound chromosomes should show this behavior. The latter

[2] I am indebted to Prof. E. B. Wilson for calling my attention to the former, and to Prof. C. E. McClung for calling my attention to the latter case.

influence seems to lie deeper in the chromosome organization. It is not revealed, however, by any morphological feature such as a difference in length or structure of the two components of a loop. Genetically one might assume the presence of balanced lethals to account for the results; but there is no evidence of their being present, and no chance of securing such evidence, because the flies are unsuitable for genetical study.

It is to be noted that the looped region of the chromosomes in *Dasyllis* is usually median. In many organisms the middle of the chromosome is apparently the last part to undergo synapsis, which suggests that the process in *Dasyllis* is actually an incomplete but otherwise normal synapsis. On the other hand, in the Diptera thus far studied, the synaptic process appears to be very different from that in other organisms, and there is no indication of synapsis beginning at the ends of the chromosomes and progressing toward the middle. Nevertheless, the fact that three pairs of chromosomes are affected similarly suggests an influence on the general synaptic process in these organisms, rather than the independent action of agents located in the respective pairs.

The chromosome behavior in *Dasyllis* recalls the genetical behavior of certain strains of *Drosophila melanogaster* in which crossing-over is greatly diminished in certain parts of linkage groups (Muller, '16; Sturtevant, '19; Detlefsen, '20). This decrease or elimination of crossing-over in part of a chromosome is just what one would expect from an incomplete synapsis such as described above.

However, it will be necessary to get both the genetical and cytological data from one organism before the evidence becomes satisfactory, and I do not wish to push the present analogy. In *Drosophila* crossing-over occurs only in the female. If this is true in *Dasyllis,* then oögenesis rather than spermatogenesis must be used for comparison. It also appears from the *Drosophila* data that many, if not most, of the cross-over modifications manifest themselves only in flies heterozygous for the cross-over "genes." In other words, as Sturtevant has suggested (p. 329), they appear to be due to an unlikeness in homologous chromosomes. In *Dasyllis* there is at present no evidence that the homologous chromosomes differ in the regions where synapsis fails to occur.

The cases involving different cross-over values in homozygous strains of *Drosophila* (C^{m} of Sturtevant, and unpublished data kindly furnished by Dr. Bridges) provide a closer analogy. At present it can only be said that the chromosome behavior in *Dasyllis* illustrates a type of mechanism which should give results similar to those observed in *Drosophila*.

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EXPLANATION OF PLATES.

All figures were drawn from sections 5μ in thickness, with the aid of a camera lucida. Magnification approximately 3,000 diameters.

PLATE I.

All figures except numbers 1 and 2 are from *Dasyllis grossa;* numbers 1 and 2 are from specimens not yet identified as to species.

FIG. 1. Spermatogonial metaphase.

FIGS. 2 and 3. Spermatogonial prophases.

FIG. 4. Very early growth stage, only one aggregate or chromosome pair represented.

FIG. 5. Same stage, representing portion of another aggregate.

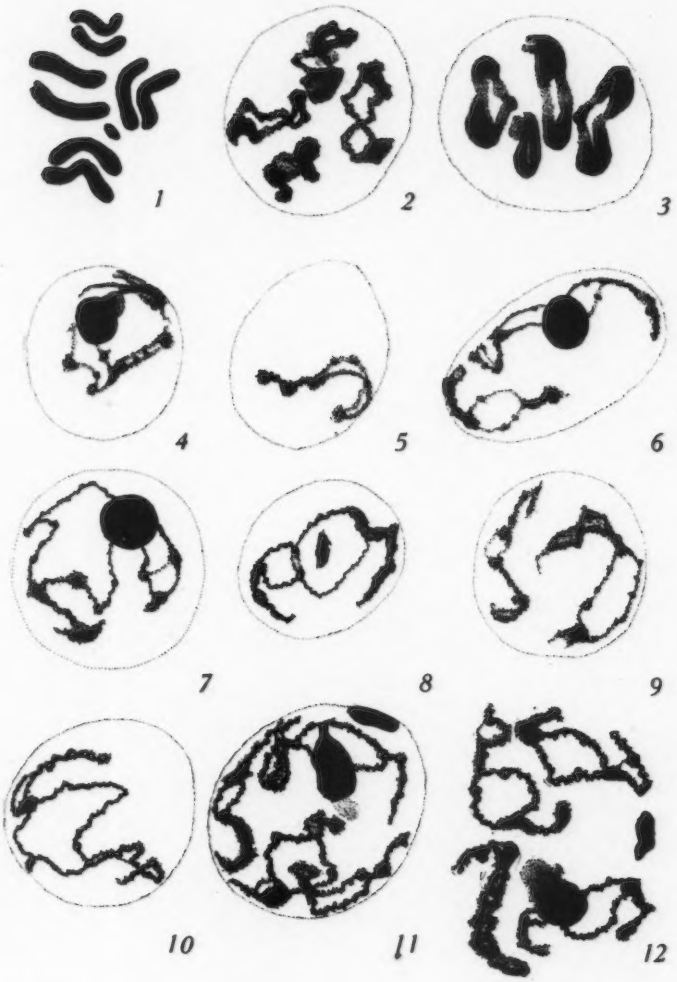
FIG. 6. About the same, or slightly later stage, showing two aggregates.

FIG. 7. Slightly later stage after the chromosomes have moved to periphery of nucleus. One chromosome pair and attached dense body represented.

FIGS. 8 TO 10. Similar stages showing other chromosomes. The chromosome pair on the left in Fig. 9 is presumably the small pair which usually has no loop or a very small one.

FIG. 11. Later stage, about middle of growth period. Entire nucleus represented, including the four pairs of autosomes and the small, condensed X.

FIG. 12. Later stage, entire nucleus; chromosomes transposed to facilitate examination.



C. W. METZ.

PLATE 2.

FIGS. 13 to 21. ♂ *Dasyllis grossa;* Figs. 22 to 28 ♀ *Dasyllis,* species not yet determined.

FIG. 13. Late prophase, first spermatocyte.

FIG. 14. Metaphase, first spermatocyte.

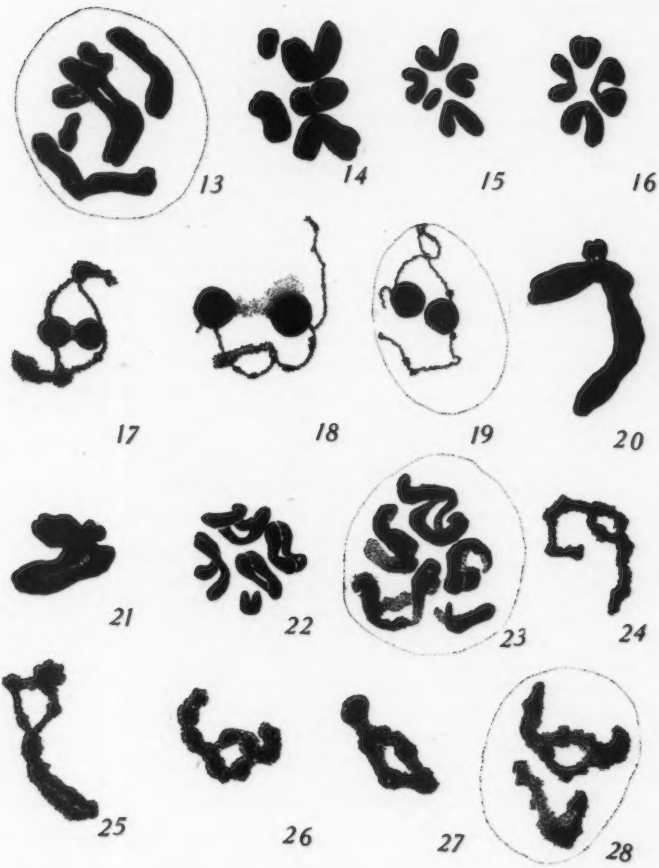
FIGS. 15 AND 16. Metaphases, two types of second spermatocytes, the former with X., the latter without it.

FIGS. 17 TO 19. Examples of the rare cases in which the dense body is represented by two separate smaller bodies.

FIGS. 20 AND 21. Examples of the chromosome pair with dense body attached, in late prophase.

FIG. 22. Ovarian cell (oögonium?), prophase showing loose association of middle region of large chromosomes.

FIGS. 24 TO 28. Chromosomes from oögonia, or early growth stage of oöcytes or nurse cells, showing median loops resembling those in the male.



C. W. METZ.

ISO-AGGLUTINATION AND HETERO-AGGLUTINATION OF SPERMATOZOA.

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I. INTRODUCTION.

The agglutination of spermatozoa reported by Buller (1900) was first adequately described by Lillie (1913). The latter distinguished two types of agglutination differing from each other in cause and in characteristics: *iso-agglutination* produced by a substance secreted by ripe ova of the same species, and *hetero-agglutination* by substances present in egg secretions and body fluids of foreign species, Lillie (1914). The characteristics of these types will be considered in the following pages. In this

report evidence is given for the first time of the occurrence of iso-agglutination in the black chiton, *Katharina tunicata,* and of hetero-agglutination between *K. tunicata* and *S. purpuratus,* and between *K. tunicata* and *S. franciscanus,* and the reciprocal hetero-agglutination between either *S. purpuratus* or *S. franciscanus* and *K. tunicata.* Similar reciprocal hetero-agglutination occurs between either *S. purpuratus* or *S. franciscanus* and *Ishnochiton magdalenensis.*

The results embodied in this report were obtained in 1920–1921 at the Hopkins Marine Station of Leland Stanford University at Pacific Grove and at the Marine Biological Laboratory at Woods Hole. I wish to express here my appreciation of the hospitality extended to investigators at the Hopkins Marine Station and my thanks to the Director, Dr. W. K. Fisher, and to Dr. Gertrude Van Wagenen, for their assistance and encouragement. The photomicrographs accompanying this paper were taken for me by Dr. Doane, of the Department of Entomology of Stanford University. For the use of a research room at Woods Hole I am indebted to the Director, Dr. F. R. Lillie, and for suggestions and criticisms to Dr. O. C. Glaser.

II. ISO-AGGLUTINATION.

1. General.

Iso-agglutination is characterized by the rapid formation of dense spherical swarms of intensely active adherent spermatozoa and by the subsequent reversal of this process, Lillie (1921). The duration of the reaction varies with the concentration and freshness of the sperm suspension and of the egg-water, Lillie (1914, 1915), and is obtained only with motile spermatozoa, Loeb (1914). This reaction has been observed in a few marine animals and wherever it occurs indicates that we are dealing with ripe reproductive cells, species true. Its significance lies in its specificity, Lillie (1921).

Iso-agglutination has been reported by Lillie (1912, 1913) for *Arbacia punctulata* and for *Nereis;* by Glaser (1914) for *Asterias forbesii;* and by Just (1919) for *Echinarachnius.* Loeb (1914) described "cluster formation" in *Strongylocentrotus purpuratus*

and in *S. franciscanus*. This, as Lillie (1921) later determined, is identical with iso-agglutination.

2. Material and Methods.

Material.—The animals used in this work represent two phyla, Echinodermata and Mollusca. Belonging to the first are *Strongylocentrotus purpuratus, S. franciscanus, Arbacia punctulata, Asterias ochracea,* and *Lepasterias æqualis;* and to the second *Katharina tunicata, Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton,* and *Abalone.*

Methods.—Every precaution is taken to prevent contamination of the gametes with body fluids. With the three species of sea-urchins this consists of washing animals and dissecting instruments with tap water, cutting around the oral disc and removing all body contents except the gonads, and then washing the cavity thoroughly with sea-water. The animals are then placed on their aboral surfaces in Syracuse watch glasses and allowed to shed their gametes through the germinal pores. A second method, suggested by Dr. O. C. Glaser, consists of washing the animals in tap water, rubbing off the spines, and drying with a towel. They are then allowed to shed as in the first method. Practically dry gametes can be obtained by this method. In the case of the starfish and molluscs the same method of sterilization is employed. The rays of the starfish are removed and the gonads, stripped from each ray, are transferred to a finger bowl of filtered sea-water and thoroughly washed. In the molluscs the gonads lie just beneath a dorsal shell and can be reached in one of two ways. The first consists of removing the ventral muscle and viscera with the exception of the gonads, and the second of removing the shell. The exposed gonads can then be thoroughly washed with sea-water, removed with blunt forceps to Syracuse watch glasses or finger bowls, and ruptured. In this manner dry gametes can be procured.

One (1) per cent. suspensions of spermatozoa are made by adding to 99 drops of filtered sea-water 1 drop of dry sperm. Pipettes known to deliver the same number of drops per c.c. are used. All suspensions are used within ten minutes or discarded. Standard egg-water is prepared by allowing one volume of dry

ripe eggs to secrete into two volumes of filtered sea-water for ten minutes. The egg-water is then separated from the eggs by filtration or centrifugation. The agglutination test is performed as follows: A drop of 1 per cent. sperm suspension placed between a slide and a cover glass supported by mm. glass rods spreads out into a thin film, and in it the spermatozoa may be studied microscopically. A fine-pointed capillary glass tube attached to rubber tubing is used for blowing drops of test solutions into the thin film of sperm suspension.

3. Iso-agglutination in Sea-urchins.

The results of my own experiments substantiate the statement of F. R. Lillie (1919, 1921) that egg secretions of *Arbacia, S. purpuratus,* and *S. franciscanus* produce three effects on species-true spermatozoa: *activation,* stimulation to increased motility followed later by a state of rest; *aggregation,* a tropistic phenomenon occurring only when there is a gradient from the egg-secretion to the spermatozoa; and *agglutination.*

Iso-agglutination Reaction.

An immediate and intense activation and the aggregation of the spermatozoa into a dense ring around the injected drop of egg-water is followed by the agglutination reaction. The ring rapidly becomes beaded in appearance and ultimately breaks up into small swarms. In each of the latter the spermatozoa are in such rapid motion that the entire swarm whirls about. Simultaneously similar smaller swarms form from the few spermatozoa trapped within the enclosed drop. For a brief period the swarms appear to rush together to form larger masses. The movements of the spermatozoa gradually slacken, and after a short interval, depending on the size of the swarms, a reversal occurs. They break up and in a short time the spermatozoa are dispersed, less active than originally.

In a special series of experiments I used spermatozoa of *Arbacia* from 25 per cent. and 50 per cent. suspensions which had stood at room temperature (21° C.) for from twelve to twenty-four hours, and were then aërated to remove the carbon dioxide, which in itself might affect the agglutination process. In 1 per cent. sus-

pensions of such spermatozoa I noticed an intermediate stage in the process of agglutination. This intermediate stage occurs during reversal. It is characterized by a radial orientation of the active spermatozoa such that the swarm momentarily takes on the appearance of a three-dimensional pinwheel. At the center of the wheel is a "nucleus" of sperm heads with tails radiating outward, while the periphery of the wheel is composed of a dense zone of sperm heads with their tails radiating inward. In optical section it is as though a set of spokes originating at the hub of a wheel were dovetailed between another set radiating inward from the rim. These pinwheels gradually break up and eventually the spermatozoa are completely dispersed as in typical reversible agglutination. This pinwheel formation is not comparable to a secondary aggregation which often follows iso-agglutination. In such aggregation the masses formed are irregular in shape; and the spermatozoa composing them are not intensely active, not oriented, and are readily dispersed by shaking.

4. Iso-agglutination in *Katharina tunicata*.

The spermatozoa of *K. tunicata* are inactive or but slightly active in sea-water. They can be roused to intense activity by foreign blood or foreign tissue extracts and exhibit a spiral method of locomotion similar to that described for other types of spermatozoa. In contact with surfaces they move anti-clockwise. This is probably due to their structure. (See Fig. 1.) As judged by their activation by various substances the spermatozoa of this species were ripe as early as April 9. Egg-water tested April 15, May 2, and May 16 failed to produce even an activation of spermatozoa. Inseminated eggs did not develop, indicating that the eggs were not ripe. On May 27 secretions from ripe eggs of females caught on the same day caused intense activation, aggregation, and a peculiar type of agglutination. The latter is comparable to the intermediate stage in the iso-agglutination reaction obtained with stale spermatozoa of *Arbacia*.

Following activation and aggregation of the spermatozoa into a dense ring, the latter rapidly break up into three dimensional pinwheels instead of into the whirling swarms characteristic of the iso-agglutination in sea-urchins and in *Nereis*. Within the in-

jected drop of egg-water where the spermatozoa are less concentrated it is possible to observe the process of formation of these pinwheels. A few spermatozoa first stick together in a group without any apparent orientation and without enough motility to produce whirling of the group. The three-dimensional pinwheels form instantly, including these clumps within the wheel. A fusion of pinwheels ensues for a short period. A complete reversal then occurs in some, whereas other pinwheels remain permanent. A protocol of one experiment will illustrate the time relations of the phenomenon.

Exp. 616.—Material: Doubly filtered standard egg-water of *K. tunicata;* fresh 1 per cent. sperm suspension of *K. tunicata.*
5–27/21.

3.15. Sperm suspension in sea-water—spermatozoa slightly active.
3.15. Inject a drop of egg-water—
Immediate intense activation.
Immediate ring formation.
3.16. Formation of small clumps of spermatozoa.
3.165. Formation of three-dimensional pinwheels.
3.17. Fusion of three-dimensional pinwheels.
3.19. Complete reversal of some pinwheels.
3.25. Decrease in activity; many permanent pinwheels remain.
3.40. No change.
5.00. No change. Spermatozoa still more active than originally.

On May 28 I obtained with animals brought in on the preceding day decided activation and ring formation, but no agglutination. In previous tests on spermatozoa of this species I had discovered that in order to obtain satisfactory results animals must be used on the day on which they are obtained. During June and July of 1921 and 1922 additional experiments were conducted for me by Dr. Van Wagenen. She reports results similar to those recorded in Exp. 616. However, complete reversal of agglutination was obtained with both standard egg-water and with the latter diluted ten times. Dilutions of $\frac{1}{100}$ and $\frac{1}{500}$ produced activation but no agglutination, thus indicating a rapid loss of agglutinating power with dilution.

5. Iso-agglutination in Other Echinoderms and Molluscs.

Iso-agglutination tests were made upon the spermatozoa of certain other Echinoderms and Molluscs: *Asterias ochracea,*

Lepasterias æqualis, Asterias forbesii; Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton, Abalone, and *Cumingia.* Both sperm and ova were ripe.

Activation occurred in every case, but no agglutination comparable to that in sea-urchins, *Nereis* or *K. tunicata.* In *Asterias forbesii, Asterias ochracea,* and *Cumingia* irregular clumps form consisting of a few spermatozoa. These, however, are irregularly dispersed and hence do not correspond to those which appear preceding typical iso-agglutination.

TABLE I.

THE EFFECTS OF EGG-WATER ON SPERMATOZOA OF THE SAME SPECIES.

	Spermatozoa.				
	Arbacia punctulata *Strongylocentrotus purpuratus* *Strongylocentrotus franciscanus* *Nereis limbata*	*Arbacia punctulata* (stale spermatozoa)	*Kalharina tunicata*	*Asterias forbesii* *Asterias ochracea* *Cumingia*	*Lepasterias æqualis* *Ishnochiton magdalenensis* *Mopalia muscosa* *Cryptochiton* *Abalone*
Motility in sea-water.....	+	+	−	−	−
Effects of egg-water:					
Activation.....	+	+	+	+	+
Aggregation.....	+	+	+	−	−
Clumping of 3–10.....	+	+	+	+	−
Dispersal uniform.....	+	+	+	−	
Dispersal irregular....	−	−	−	+	
Agglutination-swarms ...	+	+	−	−	−
Agglutination-pinwheels	−	+	+	−	−
Reversal of agglutination:					
Partial.....	−	−	−		
Complete.....	+	+	+		
Decrease in motility.....	+	+	+	+	+
Strand formation.....	−	−	−	−	−
Cytolysis.....	−	−	−	−	−

6. DISCUSSION.

The variation in character of the iso-agglutination reaction may be due in part to the degree of motility of the spermatozoa involved. Swarming is obtained with spermatozoa highly motile in sea-water; the pinwheel type with spermatozoa inactive in sea-water (*K. tunicata*), or with spermatozoa rendered less active in sea-water by staling (*Arbacia*). The fact that spermatozoa of

both *Arbacia* and of *K. tunicata* move anti-clockwise when in contact with other objects may account for the shape of the pinwheels.

Of the ten species tested in which the spermatozoa are inactive in sea-water, iso-agglutination occurred in but one species, *Katharina tunicata.*

III. HETERO-AGGLUTINATION.

1. General.

Two distinct types of hetero-agglutination were obtained in this investigation, one with sea-urchins and one with *K. tunicata.* Hence their characteristics will be considered separately and compared with other accounts of the phenomenon.

Hetero-agglutination has been reported by Lillie (1913) between *Arbacia* egg-water or blood and *Nereis* spermatozoa; by Glaser (1914) between *Arbacia* egg-water and *Asterias* spermatozoa, and the reciprocal relationship between *Asterias* egg-water and *Arbacia* spermatozoa; by Just (1919) between *Arbacia* egg-water and *Echinarachnius* spermatozoa; and by Loeb (1914) between *S. purpuratus* egg-water and *S. franciscanus* spermatozoa. Reciprocal hetero-agglutination has been reported but once, as indicated above.

Evidence is given here of hetero-agglutination of spermatozoa of *K. tunicata* by solutions of cytolyzed spermatozoa of *S. purpuratus;* also by the blood of either *S. purpuratus* or *S. franciscanus.* The reciprocal relationship is also reported: hetero-agglutination of spermatozoa of *S. purpuratus* and of *S. franciscanus* by the blood of *K. tunicata.*

2. Material and Methods.

The material and methods include those employed in iso-agglutination experiments. In addition, blood was collected and filtered and solutions of cytolyzed spermatozoa of *S. purpuratus* and *S. franciscanus* were prepared in the following manner:

A 5 per cent. suspension of spermatozoa in glass distilled water was allowed to stand at room temperature (15° C.) for one hour, shaken at frequent intervals, and filtered (through Whatman filter paper, No. 2 and No. 50) three times.[1] In order to make

[1] Solutions made from spermatozoa which were allowed to cytolyze in dis-

the solution equal in specific gravity and hydrogen-ion concentration to sea-water, concentrated sea-water and $N/100$ NaOH were added. Controls were arranged by adding to glass distilled water concentrated sea-water and $N/100$ HCl. In making the corrections I used a standard hydrometer, and a set of standards prepared by Hynson, Westcott, and Dunning for determining the hydrogen-ion concentration of sea-water.

3. Hetero-agglutination of Spermatozoa of *Katharina tunicata.*

A. Effect of Solutions of Cytolyzed Spermatozoa of S. purpuratus.

The spermatozoa of *K. tunicata,* inactive in sea-water, are intensely activated and agglutinated by solutions of cytolyzed spermatozoa of *S. purpuratus.* The reaction resembles iso-agglutination in this species in the formation of three-dimensional pinwheels and differs from it only in the irreversibility of the hetero-agglutination (Plate I.). In forty-five experiments, in which two different test solutions were employed over a period of 34 days, similar results were obtained. A single experiment will illustrate the characteristics of the reaction.

Exp. 511.—Material: *K. tunicata* spermatozoa; solution of cytolyzed spermatozoa of *S. purpuratus.*

3–21/21—2.23 P.M.

Time.	Activation.	Agglutination.	Pinwheel Formation.	Reversal.
2.23.....	Intense	0	0	–
2.24.....	Intense	0	0	–
2.245.....	Intense	Small clumps	Few and small	–
2.25.....	Intense	0	Fusion of small clusters	0
2.45.....	Intense	0	Large and numerous	0
3.05.....	Very active	0	Large and numerous	0
3.45.....	Slightly active	0	Large and numerous	0
5 00.....	Slightly active	0	Large and numerous	0

As indicated above, the formation of pinwheels occurs after a latent period of 1½ minutes in this experiment. In other experiments the latent period was often shorter, but in no case less than 20 seconds.

tilled water from 3–5 hours caused activation but no agglutination. This may be due to an unstable property of the hetero-agglutinating substance in distilled water.

Effect of Dilution on Hetero-agglutinating Power of Solutions.—Lillie (1915) found in the case of hetero-agglutinins in *Arbacia* egg-water a disproportional loss of agglutinating power with dilution, and certain preliminary experiments with one half and one fourth dilutions of solutions of cytolyzed spermatozoa of *S. purpuratus* indicated a similar loss. The results obtained with a series of greater dilutions are indicated in the following table:

TABLE II.

THE EFFECT OF DILUTION ON STRENGTH OF HETERO-AGGLUTINATION OF SOLUTIONS OF CYTOLYZED SPERMATOZOA OF *S. purpuratus*.

	Activation.			Pinwheels.		
	Dilution.	Time.	Degree.	Time.	Number.	Size.
1.....	0	Immediate	Intense	After 20″	Many	Large
2.....	1/10	Immediate	Intense	After 4′	Many	Small
3.....	1/20	Immediate	Slight	After 2.5′	Few	Small
4.....	1/30	Immediate	Slight	After 3′	Few	Small
5.....	1/40	Immediate	Slight	After 5′	Few	Small
6.....	1/50	Immediate	Slight	After 3′	Few	Small
7.....	1/60	Immediate	Slight	After 5′	Rare	Minute

In the above summary the decrease in agglutinating power appeared to be associated with a decrease in activating power of the diluted solution of cytolyzed spermatozoa. One might predict that an activating substance added to cytolyzed sperm solutions would prevent the loss of agglutinating power. This actually proved to be true, for upon the addition of an activating body a dilution of 1/60 produced as intense and immediate activation and pinwheel formation as the undiluted solution.[1]

B. Effect of the Blood of S. purpuratus and S. franciscanus.

Filtered blood of both male and female *S. purpuratus* and *S. franciscanus* causes activation and hetero-agglutination of spermatozoa of *K. tunicata* exactly like that produced by solutions of cytolyzed spermatozoa of *S. purpuratus*. The blood must, however, be taken from the animals on the day on which they are taken from their habitat. Otherwise it will produce activation, but no agglutination. This is comparable to the deterioration of

[1] The substance in question will be discussed in a subsequent paper.

spermatozoa in animals kept in the laboratory. Blood taken from fresh animals, however, retains its hetero-agglutinating power for at least three days.

With blood, as with solutions of cytolyzed spermatozoa, there is a disproportionate loss of agglutinating power with dilution.

C. Effect of the Egg-Water of S. purpuratus and of S. franciscanus.

The egg-water of ripe ova of *S. purpuratus* and of *S. franciscanus* failed repeatedly to produce either activation or hetero-agglutination of the spermatozoa of *K. tunicata.* Considering that the latter can be intensely activated and agglutinated by blood and by solutions of cytolyzed spermatozoa of these two species of sea-urchins, and in view of the striking resemblance of iso-agglutination and hetero-agglutination in *K. tunicata,* it is especially significant that the egg-water of both species of *Strongylocentrotus* fails to produce hetero-agglutination of *K. tunicata* spermatozoa. This constitutes further evidence of the specificity of the iso-agglutinating substance present in egg-waters.

D. Changes in Spermatozoa Produced by Hetero-agglutinating Substances.

In solutions which produce hetero-agglutination the heads of the spermatozoa of *K. tunicata* become swollen at the base, as indicated in Fig. 1.

A comparison of the dimensions of spermatozoa in sea-water and in hetero-agglutinating solutions will illustrate this:

Spermatozoa.	Sea-water.	Hetero-agglutinating Solution.
Head length	10 μ	10 μ
Head width at base	2 μ	2.25 to 3.35 μ

E. Discussion.

Hetero-agglutinating substances have previously been demonstrated in blood and in egg-water. Just and Lillie have suggested that in the case of *Arbacia* the hetero-active substance is a constituent of the blood, and that egg-water used was contaminated

with blood. The hetero-active substance in the egg-water of *S. purpuratus* for *S. franciscanus* is, however, not a normal constituent of the blood of *S. purpuratus*, Lillie, 1921. It is improbable that contamination with blood can account for the strong

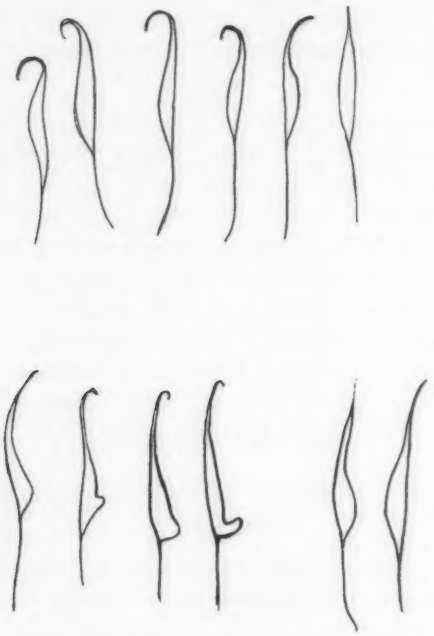

FIG. 1. Spermatozoa of *Katharina tunicata*.

a. In sea-water—normal. Mag. x 5,000 (approx.).

b. In solution of cytolyzed spermatozoa of *S. purpuratus*. The heads are swollen at the base. Mag. x 5,000.

hetero-agglutinating power of the solutions of cytolyzed sperm made from 5 per cent. suspensions of spermatozoa. Such a dilution of whole blood would have slight hetero-agglutinating properties.

It is possible that the products of cytolysis of eggs or of tissue cells of these two species of sea-urchins would also cause hetero-agglutination of spermatozoa of *K. tunicata*.

4. Hetero-agglutination of Spermatozoa of *S. purpuratus* and of *S. franciscanus* by the Blood of *Katharina tunicata.*

Since spermatozoa of *K. tunicata* were agglutinated by the blood and by solutions of cytolyzed spermatozoa of *S. purpuratus* and by the blood of *S. franciscanus,* it seemed possible that the relationship might be reciprocal. This proved to be the case, but the type of hetero-agglutination obtained resembles that described by Lillie as "mass coagulation." A single protocol will illustrate the nature of the reaction.

Exp. 585.—Blood of *K. tunicata;* spermatozoa of *S. purpuratus.* 5/4/21—2.15 P.M.

Time.	Activation.	Ring Aggregate.	Formation of		Strands.	Reversal.
			Swarms	Pinwheel.		
2.16.....	Intense	0	0	0	Few	–
2.165....	Intense	0	0	0	Many and united	0
2.25.....	Slight	0	0	0	Many and united	0

As indicated, there is no ring formation due to aggregation. The spermatozoa rapidly form strands which adhere to one another, and lose their motility. This hetero-agglutination is completely irreversible and decidedly toxic. It is of interest that the blood of *K. tunicata* which causes hetero-agglutination of spermatozoa of *S. purpuratus* and of *S. franciscanus* also causes membrane formation in the eggs of these two species. If allowed to act too long, it will induce cytolysis. A short treatment followed by a brief exposure to hypertonic sea-water will, however, lead to parthenogenetic development of the ova of *S. franciscanus,* Sampson (unpublished).

5. Hetero-agglutination in Other Echinoderms and Molluscs.

Tests were made with the spermatozoa of other Echinoderms and Molluscs to demonstrate hetero-agglutination. The results of these tests indicate two distinct types of hetero-agglutination: (*A*) the toxic "mass coagulation," described by Lillie for *Nereis* spermatozoa; (*B*) the pinwheel type, described in this report for

K. tunicata; and (*C*) a questionable third type "clumping," described by Glaser for *Arbacia.* (The "clumps" are irregularly distributed and resemble aggregation rather than hetero-agglutination.) A summary of the results is given in Tables III. and IV. These tables also include results first reported by Glaser, Just, Lillie, and Loeb, as indicated by the initials in brackets.

TABLE III.

HETERO-AGGLUTINATION OF SPERMATOZOA OF CERTAIN MARINE ANIMALS (WOODS HOLE, MASS.).

Test Solutions.	Spermatozoa.					
	Arbacia punctulata.	*Echinarachnius parma.*	*Nereis limbata.*	*Asterias forbesii.*	*Cumingia tellinoides.*	*Chiton apiculata.*
Arbacia punctulata						
Egg-water		+ *A*(J)	+ *A*(Li)	+ *C*(G)	+ *C*	–
Blood		+ *A*(J)	+ *A*(Li)			–
Sperm suspension			+ *A*(Li)			
Echinarachnius parma						
Egg-water	– (J)					
Blood	– (J)					
Nereis limbata						
Egg-water	– (Li)					
Blood	– (Li)					
Sperm suspension	– (Li)					
Asterias forbesii						
Egg-water	+ *C*(G)					
Cumingia tellinoides						
Egg-water	+ *C*					
Chiton apiculata						
Egg-water	+ *A*					
Blood						

The letters *A* and *C* refer respectively to the strand formation (Lillie, 1913) and clumping (Glaser, 1914) described in reports on hetero-agglutination.

IV. SUMMARY.

1. Iso-agglutination occurs in the black chiton, *Katharina tunicata.* This is the first report of unmistakable iso-agglutination of spermatozoa of a mollusc; also of iso-agglutination of spermatozoa which are inactive in sea-water.

2. The iso-agglutinated masses of spermatozoa of *K. tunicata* differ from those of sea-urchins and of *Nereis.* In the former the agglutinating masses resemble three-dimensional pinwheels.

TABLE IV.

HETERO-AGGLUTINATION OF SPERMATOZOA OF CERTAIN MARINE ANIMALS (PACIFIC GROVE, CALIF.).

Test Solutions.	Spermatozoa.								
	Strongylocentrotus purpuratus.	*Strongylocentrotus franciscanus.*	*Asterina.*	*Asterias ochracea.*	*Katharina tunicata.*	*Ishnochiton magdalenensis.*	*Mopalia muscosa.*	*Cryptochiton stelleri.*	*Abalone.*
Strongylocentrotus purpuratus									
Egg-water		+(Lo)	−(Lo)	−(Lo)	−	−	+C	−	−
Blood	−(Li)	−(Li)		−(Lo)	+B	+A	−	−	−
Cytolyzed sperm	−	−		−	+B	−	−	−	−
Strongylocentrotus franciscanus									
Egg-water	−(Lo)		−(Lo)	−(Lo)	−	−	−	−	−
Blood	−(Lo)	−(Lo)		−(Lo)	+B	+A	−	−	−
Asterina									
Egg-water	−(Lo)	−(Lo)							
Asterias ochracea									
Egg-water	−(Lo)	−(Lo)							
Blood	−(Lo)	−(Lo)		−(Lo)					
Katharina tunicata									
Egg-water	+A	+A		−					
Blood	+A	+A		+C	−				
Ishnochiton magdalenensis									
Egg-water									
Blood	+A	+A				−			
Mopalia muscosa									
Egg-water									
Blood							−		
Cryptochiton stelleri									
Egg-water									
Blood								−	
Abalone									
Egg-water									
Blood									−

The letters used refer to types of hetero-agglutination (*A*) to strand formation; (*B*) to pinwheel formation; and (*C*) to clumping. The latter may be considered as aggregation rather than as a type of hetero-agglutination. See text.

In these the spermatozoa are so oriented that the center consists of a nucleus of sperm heads with tails radiating outward, and the periphery is composed of a dense zone of sperm heads with tails radiating inward.

In sea-urchins and in *Nereis* the iso-agglutinating masses resemble whirling swarms in which the spermatozoa either are not oriented or are oriented with their heads forming the central nucleus of the swarm. However, a pinwheel type of iso-agglutination can be obtained with stale *Arbacia* spermatozoa. The motility of the latter is decidedly subnormal. It is possible that the variation in shape of agglutinated masses may be correlated with the degree of motility of the spermatozoa which are involved.

3. Unmistakable iso-agglutination can not be detected in any of the following: *Asterias ochracea, Asterina, Asterias forbesii, Lepasterias æqualis, Cumingia, Ishnochiton magdalenensis, Mopalia muscosa, Cryptochiton,* and *Abalone.*

4. Two types of hetero-agglutination are here reported: toxic "mass coagulation," similar to that described by Lillie (1913) for *Nereis* and by Just (1919) for *Echinarachnius,* and a pinwheel type in *Katharina tunicata,* which bears a startling resemblance to iso-agglutination in the same species.

5. Hetero-agglutination occurs between spermatozoa of *K. tunicata* and solutions of cytolyzed spermatozoa or of blood of either *S. purpuratus* or of *S. franciscanus.* The reciprocal also occurs: hetero-agglutination of spermatozoa of either *S. purpuratus* or of *S. franciscanus* by the blood of *K. tunicata.*

6. Hetero-agglutination of spermatozoa of *Ishnochiton magdalenensis* may be produced by the blood of either *S. purpuratus* or of *S. franciscanus.* The reciprocal may also be produced: hetero-agglutination of spermatozoa of either *S. purpuratus* or of *S. franciscanus* by blood of *Ishnochiton magdalenensis.*

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Loeb, J.

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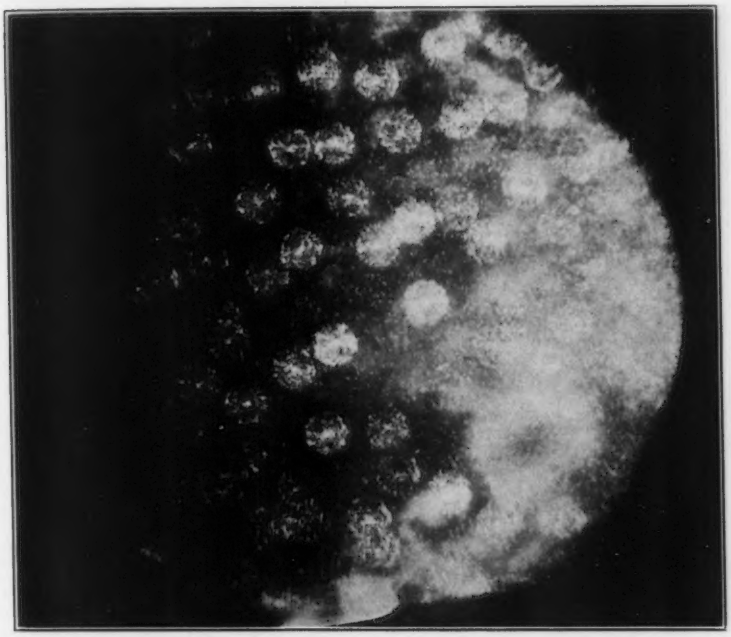
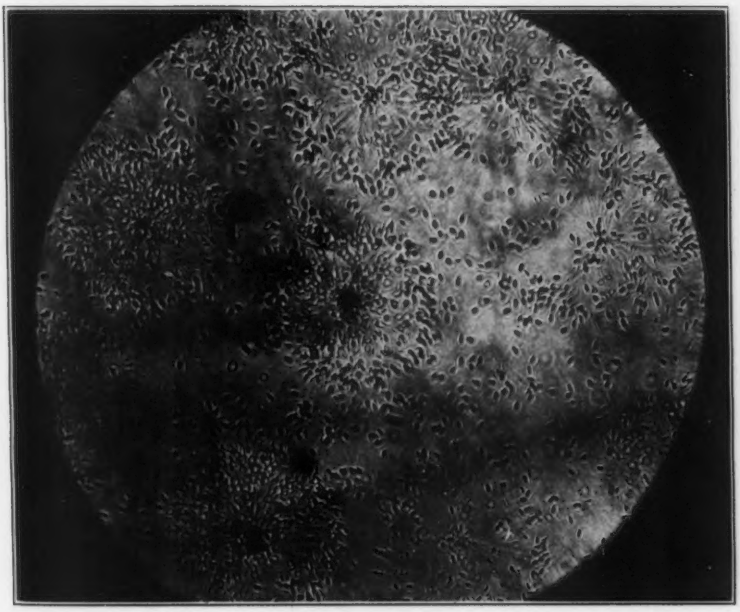
PLATE I.

Photomicrographs of Hetero-agglutination of Spermatozoa of *Katharina tunicata* in a solution of cytolyzed spermatozoa of *Strongylocentrotus purpuratus*.

The spermatozoa form three-dimensional pin-wheels.

Magnification × 60.
Magnification × 260.





M. M. SAMPSON.

ON THE PHYSIOLOGICAL PROPERTIES OF THE GONADS AS CONTROLLERS OF SOMATIC AND PSYCHICAL CHARACTERISTICS: V. THE EFFECTS OF GONADECTOMY IN THE GUINEA PIG, ON GROWTH, BONE LENGTHS, AND WEIGHT OF ORGANS OF INTERNAL SECRETION.

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To be able to interpret correctly the changes in weight of an animal incident to experimental modification of the sex-gland conditions, such as total castration or spaying followed by sex-gland transplantations, it is necessary to know the relationship of the weight of the normal to the totally castrated and totally spayed animal. Certain investigators have employed relative weights of the rat and guinea pig as indications of modification of the sexual conditions (see Steinach and Holznecht), and certainly the reactions of the guinea pig, in this respect, are not sufficiently understood to justify such conclusions as have been announced.

Steinach takes for granted that, inasmuch as the male of both rats and guinea pigs is usually heavier than the female, the testis is responsible for an internal secretion liberated by the interstitial cells that promotes growth and otherwise leads to the production of maleness, whereas the ovary is responsible for a secretion that acts to retard growth. Acting upon this assumption, he utilizes the weight of an animal at practically any age to compare with a brother or sister animal to show the differential effects of sex-gland grafts.

In 1919 the writer called attention to the work of Stotsenburg ('09, '13) on the rat wherein the growth curve was shown to be independent of the testis, but the growth curve of females was reduced by the presence of the ovary—*i.e.*, totally spayed female growth curves were from 17 per cent. to 30 per cent. higher than that of their normal sisters. It is to be understood, therefore, for the rat, that relative weight increase in a spayed female bearing a

testis graft is due to the removal of the retarding influence of the ovary and not to an influence of the testis graft as claimed by Steinach. Sufficient data were presented in the second paper of this series[1] to show that Steinach's femininized and masculinized rats can not be so adjudged on the basis of differences in weight.

Since Steinach has applied the same principles to the guinea pig to detect changes in its sexual condition after operative procedures, the writer in 1921 criticized again such indiscriminate uses of weight records and mentioned an experiment under way to determine the fundamental reactions in weight upon removal of the gonads. The present paper embodies the results obtained from this investigation.

The growth of a series of guinea pigs, consisting of normal males and females, as well as totally castrated males and totally spayed females, was followed from birth to maturity (for one year). And in addition to determining the growth curves for the four classes of animals it appeared not only desirable to examine the glands of internal secretion for possible changes correlated with sex glands, but to examine as well the effect of castration and spaying on the growth of the long bones of the leg.

II. Material and Methods.

The animals for this experiment were selected from young born from our own laboratory stock at a time when the stock was able to supply a quantity within a relatively short period of time. Comparisons in weight were then made at approximately the same time of the year, as all the animals of this experiment were born between April 20 and August 27, 1920. The forty-six animals with which the experiment was begun were grouped in the following manner: 12 normal males, 11 castrated males, 12 normal females, and 11 spayed females. These were so caged as to be recognizable at any moment, and in such a manner that pregnancy was eliminated in the normal females in all but two cases.[2]

The animals were kept in the same room, in ordinary wire cages

[1] Moore, '19.

[2] Minot, 1891, has shown that pregnancy does not cause a permanent change in the weight of a female animal; a correction for the weight of the unborn young was made in these two cases of pregnancy.

16 x 16 x 30 inches, were fed daily with carrots and hay, and usually all cages were supplied with grain (corn, oats) twice each week. Each animal was weighed on each thirtieth day from birth to the end of one year (360 days). As each weighing was made by the writer, an absence of three weeks caused one weighing of some animals to be omitted.

To avoid differences due to the operation castration was accomplished by opening the peritoneal cavity of the young male by a mid-ventral incision; the spermatic cord was tied off considerably above the testis, and by cutting the cord below the ligature the testis was removed intact. Spaying was accomplished by means of two dorsolateral lumbar incisions (one on either side) tieing, or clamping for a short time, the ovarian blood vessels, and removal of the ovary with or without a considerable amount of the oviduct. Post-mortem examination showed that in every case the entire ovary had been removed. Each animal was castrated or spayed before the thirtieth day and in the majority it was done before the age of fifteen days.

As the experiment approached its termination each animal was killed with chloroform immediately after weighing (on the 360th day) and the following data recorded: total body weight (before killing), total body length, weight of the two thyroids, hypophysis (pituitary body), the two adrenals, spleen, sex glands where present, and the lengths of the femur, tibia, and fibula.

In weighing the glands, except the hypophysis, all were rapidly removed from the animal, cleaned of superfluous connective tissue, and placed together in a ground-glass covered container, weights being obtained by difference as each gland was removed to fixation bottles. The weights were obtained as rapidly as possible, hence the glands contained whatever blood or other fluids they possessed at the moment of removal.

In handling the pituitary, the cranial cavity was rapidly opened, cranial nerves severed as the brain was lifted off the floor of the cranial cavity, and the gland very carefully dissected from under the membranes and immediately dropped into a weighing tube containing the desired fixing fluids; the weight of the container and fluid had been determined immediately before the animal was killed. In all cases the hypophysis was in the killing fluid and

weighed within thirty minutes after the administration of chloroform. In each animal thyroids, adrenals, and hypophysis were preserved in Bouin's fluid or formol-Zenker's solution for subsequent histological study.

In bone measurement the two legs of each animal were stripped of muscle and boiled in a weak alkali solution for a short time, after which they were cleaned of all muscle and ligaments. They were then measured by means of graduated calipers (grad. to .01 cm.) and the length of the two corresponding bones averaged. Second measurements were made at a later date by Mr. N. F. Fisher, who was unacquainted with my measurements, and the two sets compared; in case of differences a third measurement was taken.

III. Growth Curves.

The weights of each animal of the experiment on each thirty days is given in Table I., and the growth curves for the four groups are shown in Fig. 1. These curves were constructed from an average of the weights of all animals in each of the four groups for each weighing period—*i.e.*, 30, 60, 90 days, etc.

Table I.

Showing Individual Weights of Each Animal up to 360 Days and the Averages for Each Group as a Whole at Each Weighing Period.

Animal.	30 da.	60 da.	90 da.	120 da.	150 da.	180 da.	210 da.	240 da.	270 da.	300 da.	330 da.	360 da.
Normal Males												
X1.....	172	325	500	—	680	700	760	810	865	870	930	980
X2.....	205	305	440	640	—	740	770	825	830	885	895	835
X3.....	180	330	495	—	630	670	720	765	815	855	807	870
X4.....	162	270	450	625	—	730	805	855	930	980	980	967
X5.....	120	200	330	—	440	475	520	520	580	600	450	575
X6.....	210	375	580	—	680	715	770	810	825	890	970	950
X7.....	255	405	505	580	520	650	705	725	790	810	775	810
X8.....	340	540	—	725	830	930	980	1,065	920	1,040	1,115	1,120
X9.....	245	455	—	630	700	755	720	665	670	685	660	650
X10.....	155	295	—	425	490	500	535	555	585	605	650	550
X16.....	280	—	465	540	595	620	660	725	725	785	860	885
X22.....	—	510	560	670	810	920	1,040	1,080	1,150	1,220	1,195	1,280
Average..	211	364	480	604	637	700	749	783	807	852	857	872

Castrated Males

X11.....	135	245	—	365	410	490	510	540	565	600	600	555
X12.....	—	450	—	560	615	650	750	dead	—	—	—	—
X13.....	—	445	—	545	585	575	605	700	755	795	810	850
X14.....	165	285	400	465	535	615	690	735	800	835	870	940
X15.....	370	515	605	635	700	740	765	825	855	dead	—	—
X17.....	280	—	470	525	595	635	650	720	748	dead	—	—
X18.....	240	—	410	440	510	565	640	685	715	750	795	830
X19.....	270	—	450	500	580	640	725	770	dead	—	—	—
X20.....	—	365	415	415	510	555	530	585	580	620	655	695
X21.....	—	360	455	545	570	650	dead	—	—	—	—	—
X23.....	—	415	470	605	735	740	845	907	925	940	1,015	995
Average..	243	385	459	509	576	623	671	718	742	756	791	810

Animal.	30 da.	60 da.	90 da.	120 da.	150 da.	180 da.	210 da.	240 da.	270 da.	300 da.	330 da.	360 da.

Normal Females

Y1.....	175	305	460	—	640	670	740	700	700	700	730	807
Y2.....	285	370	510	645	—	760	800	875	900	945	980	880
Y3.....	197	265	475	620	—	770	780	815	855	865	855	925
Y4.....	170	210	390	515	—	640	675	750	770	805	875	875
Y5.....	130	245	360	—	590	dead	—	—	—	—	—	—
Y7.....	180	390	615	—	670	720	760	dead	—	—	—	—
Y8.....	222	380	—	590	640	665	740	785	790	815	870	900
Y9.....	215	360	—	550	590	645	705	590	670	760	815	800
Y10.....	250	390	—	580	620	685	720	780	835	850	830	890
Y19.....	—	390	435	520	625	640	685	745	795	855	900	975
Y20.....	—	335	370	435	510	540	585	620	655	700	730	785
Average..	202	330	452	557	610	673	719	740	774	810	843	871

Spayed Females

Y11.....	240	390	—	605	660	735	780	840	870	905	dead	—
Y12.....	370	530	—	680	735	740	745	760	835	917	930	995
Y14.....	163	280	375	455	515	593	645	700	765	815	850	915
Y15.....	300	400	485	550	590	580	645	720	700	740	715	700
Y16.....	262	—	440	490	565	620	630	655	680	740	810	850
Y17.....	—	330	365	435	510	560	575	625	650	695	755	800
Y18.....	—	345	390	460	520	570	595	630	655	695	755	800
Y21.....	—	395	510	590	630	695	dead	—	—	—	—	—
Y22.....	—	415	470	575	680	735	795	845	835	890	920	925
Y23.....	—	470	490	520	625	700	690	803	850	935	1,020	1,045
Average..	267	395	440	536	603	652	677	730	760	815	844	879

Examining the growth curves one sees that each group as a whole ascends in an unbroken curve from the first weighing to the last one made on the 360th day. A few animals of each series showed individual temporary loss in weight, but as a group the increases from month to month were continuous.

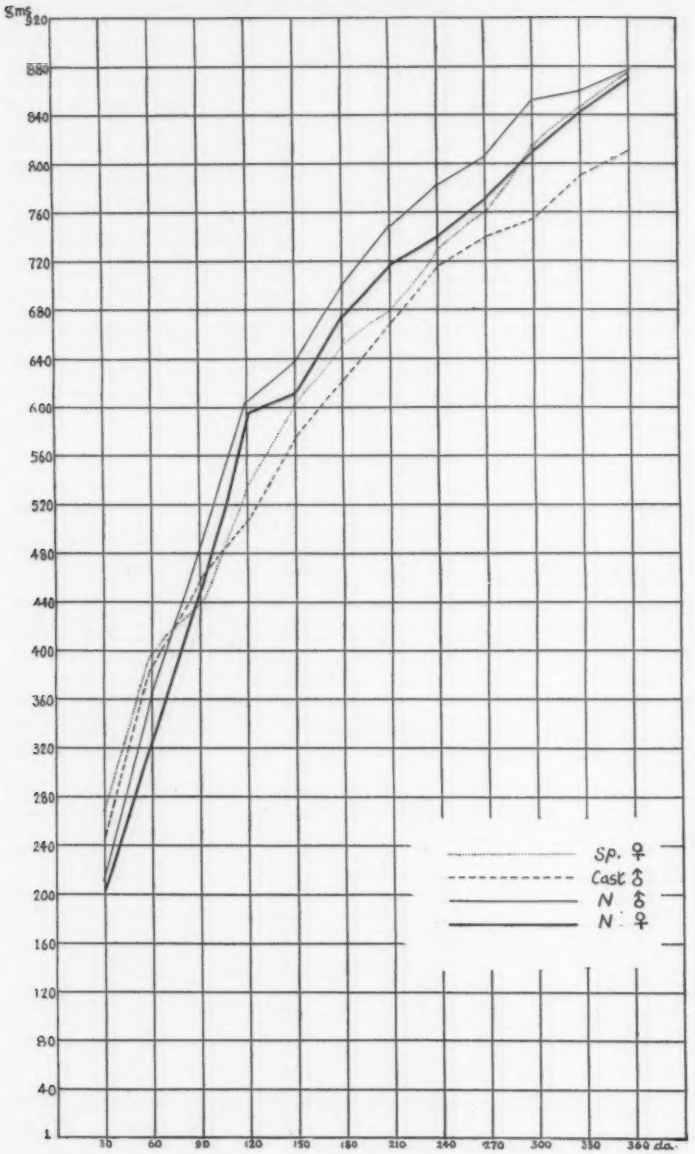

FIG. 1. Growth curves for normal males (*N*♂), normal females (*N*♀), totally castrated males (*Cast* ♂), and totally spayed female (*Sp* ♀), guinea pigs for one year from birth. The curves were constructed from the average weight of each group, on 30, 60, 90 days, etc., as given in Table I.

In reference to growth curves, as well as to all other elements of the investigation, three particular points have been kept in mind: (1) the possible demonstration of sexual differences, (2) the effects conditioned by total castration, and (3) the effects conditioned by total spaying.

Sexual Differences.—Comparing the growth curves of the normal animals, male with female (Fig. 1), one sees that the males are constantly the heavier up to the end of the first year of growth, when the average weight of the two groups is the same. This agrees with the findings of Minot ('91), who demonstrated that the male guinea pigs were heavier than the females (as a group) up to about the end of the first year, after which the female growth curve crossed that of the males, and from this time on the females were from 1 per cent. to 7 per cent. heavier than the males.

Total Castration.—From a glance at the growth curves in Fig. 1, or reference to the average weights from both groups of animals as seen in Table I., it becomes apparent that castrated males are lighter in weight than normal males at all ages from ninety days to the end of one year. In other words, castration causes a relative loss in weight in the guinea pig when compared with the normal male.

Total Spaying.—Spayed females also exhibit a relative loss in weight when compared with the normal females. The young spayed females at the beginning of the experiment were slightly heavier than the normal females, due merely to chance variation in weight in the selection of the animals, but at the age of ninety days the normal females are slightly heavier and continue so throughout the experiment until the 360th day, when the spayed females averaged approximately 1 per cent. heavier than the normal females. One is forced to conclude from this data that spaying results, for a certain period, in a decreased body weight (compared with the normal females), but that this loss is made good at a later time.

Discussion.—The particular point in mind for solution was whether the weight of an animal could be depended upon to offer an index of its sexual condition in case of sex-gland transplantations. As mentioned above, Steinach has placed considerable emphasis on the differences in weight after castration and spaying

and subsequent transplantations of sex glands of the opposite nature. To mention specific data, Steinach and Holzknecht ('16, pp. 493 and 495) publish the following weights: (*a*) normal male, 980 grams; normal female, 808 grams; femininized males, 516 grams; and (*b*) normal female, 845 grams; normal male, 1,002 grams; masculinized female, 1,200 grams. Since the above writers believe that an ovary graft femininizes an animal, the data of (*a*) theoretically shows that the ovary graft has so functioned as to reduce the weight of the male to such an extent that it is 47 per cent. lower in weight than had it continued a normal male, and is indeed 36 per cent. below the weight of a normal female; thus the ovarian grafted male is more female than the normal female herself. Also, since a testis graft masculinizes an animal, the data of (*b*) indicates to them that the female, as a result of the testis graft, has gained 42 per cent. in weight above what it would have been had it remained a normal female, and to have increased in weight 19 per cent. above the normal male; thus, here also, the animal is considered much more a male, as an after effect of the testis graft, than the normal male.

Instead of showing that the ovary has a decided effect in lowering body weight, my own results on the guinea pig show that females deprived of the ovaries are lighter in weight up to the end of the first year than those possessing ovaries. While it is true the normal males are heavier than castrated males (indicating, possibly, that the testis promotes growth), nevertheless the averages of the two groups throughout the year give a difference of but 7.6 per cent. in favor of the normal males, as against the tremendous differences in the case of Steinach and Holzknecht. Comparisons of animals of the same litter do not afford more convincing arguments in favor of weight comparisons. One example being sufficient, attention is directed in Table I. to females Y19 and Y20. These two animals of the same litter (sisters) were kept in the same cage throughout their entire life and hence possessed equal chances of growth; each suffered no reversals, but continued to increase consistently for the 360 days, yet Y19 maintained a weight from 15 to 20 per cent. above Y20 throughout the entire period of observation. Let us suppose that Y19 had been spayed and received a testis graft at an early age. Comparisons

would have shown that the animal had increased 20 per cent. in weight, as a result of the experiment, even had the testis not grown.

Without further discussion of the above point, it should be clearly demonstrated to the reader that such a comparison of weights of two or three animals, chosen at random, is absolutely unreliable as evidence of their sexual nature. As the writer has pointed out previously, the same criticism applies to rats. Since the fallacy of such comparisons can not but be realized, it follows that as evidence of masculinization and femininization such data should be ruled out when considering the value of the various types of data presented to support this idea.

Turning to the literature for actual data supporting the well-established idea that castration or spaying causes an overgrowth in body weight in animals (particularly the former), one realizes that there are indeed few experiments adequately controlled that give results supporting this idea.

Stotsenburg is apparently the first to carry out experiments on mammals determining the effects of gonadectomy wherein a sufficient number of animals were utilized to warrant definite conclusions. Reference was made above to these results. He found that the growth curves of normal and castrated male rats were very similar, and that castration in the rat did not result in a relative increase in weight from birth to maturity. In spayed females, however, there was a decided relative increase in weight as compared with normal females; the increase amounted to from 17 per cent. to 30 per cent. above the normal.

Hatai ('13, '15) confirms in general the above conclusions of Stotsenburg, though in a late paper he is inclined to believe that castration of male rats causes a slight relative increase in body weight over the control males; this, however, is not more than 3 per cent. to 5 per cent. And in spayed females there is both an increase in relative body weight and body length.

Livingston ('16) studied the effect of castration and spaying in the rabbit, but it is difficult to properly interpret his data, since the relative ages of his animals were unknown. Furthermore, he states that at the time of operation the ages varied from a few weeks to about one year, and that body weights ranged from 300

grams to more than two kilos, yet averages were taken on the group as a whole. In his Fig. 7 (curves 13 and 14), however, in which normal males controlled operated males and normal females the operated females from the same litter (ages not given), the indications are that the castrated males increase in weight, compared with the normal males, for a period of four months after operation. One is unable to judge, however, the comparative ages of all concerned and undoubtedly this would make a considerable difference in the results. This writer, as well as Hatai, believes that after castration there may or may not be a hypertrophy of the hypophysis, depending upon unknown factors; if hypertrophy does not take place, the animal undergoes a relative increase in weight, whereas there is an absence of a relative body weight increase if the hypophysis does not so react. If this is later proven to be correct, one will be unable to properly interpret weight differences following sex-gland removal without considering the possibilities of secondary influences dependent upon the hypophysis. One is led to believe from the experiments of Livingston with the rabbit that totally spayed females are hindered in growth; his curves show the normal female weight to be above that of the spayed females.

Thus Stotsenburg and Hatai are in practical agreement that the testis does not influence the growth of the rat, whereas in the rabbit Livingston believes that the testis retards growth; castrated animals are heavier than controls. My own experiments show but little effect in the guinea pig from castration, though there is a slight indication that the testis stimulates growth. As to the effects of the ovary on growth, Stotsenburg and Hatai agree that elimination of the rat ovary results in relative increases in the growth curves; Livingston, however, believes that ovariotomy lowers the growth curves in the rabbit. In the guinea pig my experiments show a slightly reduced growth curve for the spayed females up to the end of the first year.

IV. Body Length of Normal and Operated Animals.

The total body length of each animal was determined as each was killed on the 360th day, and it appeared desirable to record these observations for the bearing they may have on growth in

general. The body length of each animal is given in Table II., as well as the average lengths for the different groups as a whole. From the average of each group one sees that the groups have essentially the same relationship to each other as the average weight determinations—*i.e.*, in the order, normal males > normal females > spayed females > castrated males. This indicates that the weight as a whole is due to a general growth rather than in certain groups to excess fat deposition as a secondary result from some specific action of the glands of internal secretion.

V. Weight of the Hypophysis.

According to many investigators there is a more or less specific effect upon the hypophysis from gonadectomy; however, many of the conclusions found in the literature are diametrically opposed. It appears that too often merely gross weight comparisons of this small gland from two animals are used rather than a comparison of percentages of body weight; where small numbers of animals are used the latter method would offer more adequate means of studying specific reactions of the gland.

Table II.

Showing Individual Weights of Organs of Internal Secretion and Lengths of Bones and the Averages for the Same for the Various Groups.

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.
					Normal Males					
X1.....	980	31.6	.0116	.1646	.7300	1.0084	4.64	5.01	4.16	4.4406
X2.....	835	30.0	.0192	.2116	.8118	1.0225	4.62	5.08	4.30	3.9619
X3.....	870	30.4	.0152	.1642	1.1260	.6178	4.57	4.855	4.15	3.7612
X4.....	967	32.9	.0192	.1710	.7419	.6899	4.77	5.135	4.35	5.3212
X5.....	575	29.5	.0136	.1088	.5208	.7012	4.525	4.80	4.045	2.0410
X6.....	950	30.9	.0072	.1421	.9566	1.1141	4.51	4.95	4.100	5.9152
X7.....	810	27.5	.0144	.1402	.8900	.9570	4.46	4.87	4.16	4.6440
X8.....	1,120	32.0	.0150	.1886	1.3630	.9094	4.715	5.13	4.28	6.5590
X9.....	650	29.7	.0116	.1270	.6872	.5485	4.465	4.86	——	2.8073
X10....	550	27.0	.0104	.1330	.7433	2.9542	4.37	4.70	3.67	2.4573
X16....	885	30.6	.0147	.1306	.6580	.7095	4.645	5.00	4.24	4.2510
X22....	1,280	33.0	.0170	.1400	.7152	1.1344	5.095	5.61	4.70	5.0086
Average.	872	30.4	.0140	.1518	.8286	1.0305	4.615	5.00	4.196	4.2640

Castrated Males

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.
X11....	555	28.0	.0018	.1546	.5200	.4034	4.4	4.765	——	
X13....	850	29.0	.0121	.1829	.7595	.8134	4.495	4.855	4.10	
X14....	940	28.5	.0154	.1826	.7100	1.6100	4.40	4.85	4.135	
X18....	830	29.0	.0130	.1208	.8598	.7920	4.45	4.95	4.205	
X20....	695	28.0	.0141	.1786	.7154	.8522	4.475	4.875	4.140	
X23....	995	29.5	.0170	.1700	.9584	.9516	4.780	5.185	4.380	
Average.	810	28.6	.0122	.1649	.7538	.9037	4.5	4.915	4.192	

Normal Females

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.
Y1.....	807	29.7	.0165	.2180	.7054	1.3174	4.37	4.70	3.93	.1302
Y2.....	880	30.1	.0035	.1610	.7793	.7526	4.47	4.92	4.195	.1918
Y3.....	925	29.2	.0142	.1372	1.0010	1.3911	4.375	4.725	4.015	.1766
Y4.....	875	29.1	.0164	.1710	.5230	1.3171	4.31	4.68	4.01	.1434
Y8.....	900	30.6	.0124	.1246	.6050	.7596	4.49	4.95	4.21	.1294
Y9.....	800	29.5	.0174	.1165	.7721	1.0550	4.47	4.945	4.265	.1784
Y10....	890	29.3	.0162	.1204	.5314	.9710	4.455	4.78	4.06	.1050
Y19....	975	29.0	.0170	.1873	.7169	.9610	4.57	4.95	4.22	.2158
Y20....	785	29.0	.0166	.1742	.5708	1.3666	4.575	4.865	4.125	.1552
Average.	871	29.5	.0150	.1567	.6894	1.0990	4.454	4.835	4.114	.1584

Spayed Females

Animal No.	Body Wt., Grams.	L'gth. Cm.	Hypophysis, Grams.	Thyroids, Grams.	Adrenal, Grams.	Spleen, Grams.	Femur, Cm.	Tibia, Cm.	Fibula, Cm.	Sex. Gl., Grams.
Y12....	995	29.5	.0144	.2146	.6645	1.2505	4.61	5.14	4.345	
Y14....	915	29.0	.0134	.2096	.6407	1.1181	4.30	4.8	4.100	
Y15....	700	29.2	.0142	.1650	.5416	1.1551	4.595	5.15	4.300	
Y16....	850	29.2	.0164	.1718	.5418	.8070	4.68	5.06	4.350	
Y17....	800	27.0	.0128	.1412	.5286	.8470	4.50	4.91	4.220	
Y18....	800	28.5	.0119	.1534	.3540	.9126	4.55	4.95	4.240	
Y22....	925	29.0	.0118	.1498	.5760	.8744	4.56	5.05	4.300	
Y23....	1.045	29.5	.0124	.1348	.6456	.7556	4.70	5.07	4.345	
Average.	879	28.8	.0134	.1675	.5616	.9625	4.562	5.01	4.275	

In my own experiment the average gross weights of several glands from animals of the same age, sex, and operated condition form the basis of the comparison, the average body weights of the same lots of animals being known. Table II. gives the weight of individual hypophyses as they were removed from the animal on the 360th day of life. The weights of all the hypophyses for each group were averaged and a comparison of these average weights should give as good an indication of the reactions, if any, as any method of comparison that could be used.

Sexual Differences.—Comparing the average weight of the twelve normal male hypophyses (.0140 gram) with the average of nine normal female glands (.0150 gram), we see that the female hypophysis is 7.1 per cent. heavier than that of the male. And comparing the average body weights of the same two groups, one sees a difference of but one gram; in other words, the average body weights of the two groups are almost identical. There appears, therefore, to be a slight sexual difference in the weight of the male and female guinea pig hypophysis at the end of one year's growth, the female hypophysis being slightly heavier than the male.

Castration.—Comparing the average weights of the twelve normal male hypophyses (.0140 gram) with the average of the six castrated male hypophyses (.0122 gram), we find that the normal male hypophysis is 14 per cent. heavier than the hypophysis of castrated males. Comparing the body weights of these two groups, the normal males are 7.6 per cent. heavier than the castrated males, or, in other words, the relationship of the hypophysis of the normal male to that of the castrated male is proportionally greater than the relationship of the body weights. Thus we conclude that castration causes a relative decrease in the hypophysis as well as an actual one, as shown by the figures in the table.

Spaying.—If one compares the average weight of the hypophyses of the nine normal females (.0150 gram) with the average weight of the same gland of the eight totally spayed females (.0134 gram), it will be seen that the normal female hypophysis is 12 per cent. heavier than the hypophysis of the spayed females. And looking at the ratio of body weights of the two groups, it will be seen that the spayed females are approximately 1 per cent. heavier than the normal females. Thus at the end of one year the normal female hypophysis is both relatively and actually heavier than that of the spayed females, or, in other words, total spaying causes a decrease in weight of the hypophysis.

Discussion.—It has been impossible to find in the literature more than one or two observations wherein the weight relations between the hypophysis of the normal male and female of any group of animals have been determined. It would appear that a knowledge of sexual differences would be of great value in attempting to

unravel the existing relations between the sex glands and the hypophysis. Hatai ('13) has shown that the hypophysis of the female albino rat is more than twice the weight of that of the male, and that in the Norway rat weight difference is also constant, but of less degree than in the albino variety. As noted above, the female guinea-pig hypophysis was heavier than the male gland, though the difference in this case is but 7 per cent. Too few determinations have been made, however, to warrant the conclusion that the same result, in general, would hold for other animals.

There is a great amount of controversial evidence on the effects of gonadectomy on the hypophysis, not only among the different groups of animals investigated, but also for the same group investigated by different workers. Fischera ('05) maintained that the hypophysis increased very materially in weight when gonadectomy was performed on the common fowl, cattle, rabbit, guinea pig, and buffalo. Marrassini and Luciani ('11), however, denied an increase in the hypophysis under these conditions for practically the same group of animals utilized by Fischera. And since the publications of the latter an increase in the hypophysis following gonadectomy has been reported for the female dog and rabbit (Parhorn and Goldstein, '05), male dog and rabbit (Cimorini, '08), male and female rat (Hatai, '13); but an increase of the hypophysis has been denied for the male guinea pig (Pirsche, '02), the male of the common fowl, cattle, dog, rabbit, guinea pig, sheep, and the female guinea pig (Marrassini and Luciani, '11). These very different results are undoubtedly due to inadequate controls. It is very difficult to judge, in most instances, if the conclusions drawn are warranted by the materials considered; even if they are, the majority of observations have been made upon an inadequate number of animals, many times even upon animals of a different stock. It appears that the observations of Hatai stand almost alone in respect to the materials used, for in this investigation the growth relations are sufficiently understood and conducted upon a sufficient number of animals to warrant an adequate comparison.

Without attempting to discuss the ideas of the various writers, advanced as explanations of their own particular results, I will

but mention a correlation between the sex glands and the hypophysis first mentioned by Hatai and approved by Livingston. Hatai called attention to an apparent reciprocal relationship between these two glands, of such a nature that different effects can be noted in subsequent growth. He noted that castrated animals showing an hypophyseal hypertrophy did not show an overgrowth in body weight, and conversely if the hypophysis had not increased in weight, relative to the normal, the animal did show an overgrowth in body weight. "Thus if a compensatory growth of the hypophysis does not follow, as is the case after spaying, the product of the unaltered gland must be employed for two purposes: one, to replace the ovarian hormone, and, two, for the normal uses, whatever they may be."

Later experiments led Hatai to believe that his assumptions were correct, as further observations substantiated the idea. Livingston ('16) agrees with the assumption of Hatai inasmuch as the results of his series of gonadectomized rabbits could be interpreted on this basis. He states that when the body responds by an increase in weight, even though slight, the pituitary does not show a compensatory hypertrophy.

My own observations on the guinea pig, however, do not accord with the idea that in the absence of hypophyseal hypertrophy an overgrowth of body proportions follow. The average weight of the normal male hypophysis was 14 per cent. greater than that of the castrated male, whereas the average body weight of the castrated males was less than that of the normal animals; and similarly the average weight of the normal female hypophysis was 12 per cent. greater than that of the spayed females; and, again, the average total body weight of the spayed female was below, or almost identical with, that of the normal female. And in each of the above cases, to substantiate the hypothesis, there should have been a relative increase in the body weight of the gonadectomized animals, since the hypophyseal weight was less. It remains, therefore, for subsequent investigation to prove that there is this general relationship that has been indicated by the rat and rabbit material.

VI. Growth of Thyroids.

The actual weights of the thyroid glands of each animal of the series are shown in Table II. and the average weight of the glands of each group as well (both thyroids weighed in each case).

Sexual Differences.—When the average weights of the twelve normal male thyroids (.1518 gram) are compared with the average weights of the nine normal female glands (.1567 gram), we see that the normal female thyroids are approximately 3.2 per cent. heavier than those of the normal males. Since the total body weights of the two groups do not vary more than one gram, it appears that the female guinea-pig thyroids are heavier than the male glands.

Castration.—Comparing the average thyroid weights of the normal males (.1518 gram) with those of the castrated males (.1649 grams), the thyroids of the castrated males appear to be 8.6 per cent. heavier than those of the normal animals. But since the body weight of the normal males is 7.6 per cent. greater than that of the castrated males, the relative differences of thyroid weights are really considerably greater than the differences of their actual weights. Hence we may conclude that castration in the guinea pig favors the growth of the thyroids.

Spaying.—The average weight of the normal female thyroid (.1567 gram) compared with the average of the spayed females (.1675 gram) reveals a difference of 6.8 per cent. in favor of the spayed animals. Since the averages of body weight favor the spayed females by only 1 per cent., we see that elimination of the ovaries favors growth of the thyroids in the females.

To the writer's knowledge there is no authenticated observation of a sexual difference in the weight of the thyroid gland in any animal. It must be emphasized that results obtained from a comparison of one or two animals are decidedly unreliable and of little value.

In all cases where a sufficient number of animals of corresponding ages have been compared the variability in weight is such that results of a definite character are not indicated. Thus Livingston ('16) determined the weights of the thyroid glands in a large number of rabbits, normal and operated, but the degree of weight

variability was so pronounced that no definite conclusions were warranted. Hatai ('18) definitely states that there is no apparent sexual difference in the weight of the thyroids in the rat. Furthermore, the variability in thyroid weight was so pronounced among the operated rats that results of a definite character were not indicated.

In the guinea pig comparison of the average weights of male and female thyroids at the end of one year favored the females to a slight extent.

Gonadectomy apparently favors the growth of the thyroid in the guinea pig inasmuch as each operated group showed a definite increase in the thyroids at the end of one year. However, in view of the many failures to establish a definite effect, one must be very skeptical indeed in applying these results as a general effect of gonadectomy. Obviously repetition of the experiment is necessary before general principles can be held as established even for the guinea pig.

VII. Growth of Adrenals.

The individual weights of the adrenals (two in each case) can be seen by reference to the different groups given in Table II.

Sexual Differences.—When one compares the average weight of the normal male adrenals (.8286 gram) with the average weight of the normal female adrenals (.6894 gram), it is apparent that there is a considerable difference in the two sexes; the normal male adrenal weights are approximately 20 per cent. greater than those of the normal females, the average of the body weights of the two sexes being almost identical.

Castration.—The average normal male adrenals (.8286 gram) compared with the average castrated male adrenal (.7538 gram) is 9.9 per cent. heavier. However, when average body weights are compared, one sees that the normal male is 7.6 per cent. heavier; the relative difference, therefore, is seen to be very small, though favoring slightly the normal animals. It appears, therefore, that castration inhibits the growth of the adrenals to a slight extent.

Spaying.—When the average weights of the normal female adrenals (.6894 gram) are compared with the average weights of

the spayed female adrenal (.5616 gram), one sees that the normal female adrenal is approximately 22.7 per cent. heavier than those of the spayed female. Since the body-weight comparison shows a difference of but 1 per cent., the conclusion is that spaying is followed by an inhibition of the growth of the adrenal gland.

Aside from the present data for the guinea pig there is apparently but one other set of data that gives an adequate comparison of the suprarenal weights in the two different sexes. Livingston's data ('16), consisting as it does of suprarenal weights at different ages and conditions of life, is inadequate for an intelligent comparison. Hatai ('15), however, found for the rat a marked sexual difference in the weights of the suprarenal glands. The female glands are approximately double those of the males in the mature animals. For the guinea pig there is also a decided sexual difference in the suprarenal weights, but in these animals it is the male that is the larger. At the end of one year the male suprarenals are approximately 20 per cent. greater in weight than those of the female.

Certainly to understand the general relationship existent between the suprarenals and the sex glands more data are desirable; particularly is this true for the guinea pig, in which the number of animals is inferior to that of the rat. Obviously it is useless to attempt an analysis of the conditions until the facts are well established.

One may well conclude that the same is true for the effects derived after gonadectomy, and particularly so in view of the many discrepancies noted in the literature. The fundamental conditions of the various experiments are so varied that only divergent results can be anticipated. Soli ('09) reports a decrease in the relative weight of the suprarenals after castration in guinea pigs, rabbits, and chicks, but the number of animals observed was small. Marrassini and Luciani ('11) reported considerable data on the suprarenal weights in both sexes of the rabbit and guinea pig after gonadectomy, but in their experiments the time limit was too brief for complete changes to have been registered. Castrated and spayed males and females were compared with normal animals of approximately the same age, but the operated animals were killed in all cases within ninety days after the operation, and sev-

eral were allowed to live but fifteen days after gonadectomy. The majority of the cases compared indicate a tendency toward a suprarenal hypertrophy in both sexes of the rabbit and guinea pig. Hatai ('15) found that the suprarenal of the male rat increased materially in weight after castration, whereas the female suprarenal showed a decrease of from 5 per cent. to 25 per cent. compared with normal females. In the guinea pig the relative weight of castrated and normal male suprarenals in my experiments are but little different. It appears, however, that there is a slight reduction in the weight of these glands in the castrated animals. In the spayed females the suprarenals were approximately 22 per cent. lighter in weight than those of the normal females.

The fundamental relationship between the sex glands and the suprarenals may be actually different for different types of animal forms, as is indicated by the discordant results reported. But it is undeniable that more exact data are necessary before an approach to the truth can be formulated. For the experimental data to be of value comparisons must be made with animals of similar ages and existing under similar living conditions. It is obvious, also, that data based upon considerable numbers of operated cases are of much greater weight than that obtained from a random comparison of a few animals.

VIII. Growth of the Spleen.

While the spleen is not ordinarily considered an organ of internal secretion, it appeared desirable to include the weights of this organ in the various animals, as all such data may prove of value at a later time.

Sexual Differences.—Comparing the average weight of the normal male spleen (1.0305 grams) with that of the average of the normal female spleen (1.0990 grams), one sees that the normal female spleen is 6.6 per cent. heavier than that of the male; since the average body weights are the same, we may conclude that there is a slight sexual difference in the spleen favoring that of the female.

Castration.—Comparing the average weight of the normal male spleen (1.0305 grams) with the average weight of the castrated male spleen (0.9037 gram), a slight difference is evident. The

normal male spleen is 14 per cent. heavier than the castrated male spleen, but the body weight of the normal male is 7.6 per cent. greater than that of the castrated male; the relative difference is therefore not so great. Apparently castration slightly inhibits growth of the spleen.

Spaying.—Comparing the normal female spleen with that of the spayed female spleen, we note that the spayed female possesses on the average a spleen 14 per cent. less in weight than the normal; since the body weights are but slightly different, we may conclude that spaying results in a decrease in the spleen growth.

IX. Bone Lengths.

Reported increases in the length of bones following gonadectomy indicate that the sex glands control to some degree the lengths acquired. In order to determine possible differences referable to a sex-gland disturbance, the bones of the hind leg of each animal of the series were measured at the time they were killed. These measurements are given in Table II. and are expressed in average lengths of the two similar bones from each animal. Thus the given femur length for each animal is the average between the lengths of the two femurs, etc., and it has been found that differences between the two bones are often greater than could be anticipated.

Sexual Differences.—When a comparison was made between average lengths of the hind-leg bones of the normal male group and an average of similar bones from the normal female group, it was found that the bones of the normal males were but slightly greater in length than those of the females. The difference was as follows: femur, 3.6 per cent. longer; tibia, 3.4 per cent. longer; fibula, 1.9 per cent. longer.

The sexual difference in bone lengths of normal guinea pigs is therefore one of slight degree, the males being favored as to growth in length.

Castration.—When the average lengths of the bones of the normal males were compared with similar ones of the castrated animals, the actual lengths were greater in the normal animals than in castrated ones; the femur was 2.5 per cent. greater in length, the tibia 1.7 per cent., and the fibula considerably less than 1 per

cent. greater in length than the corresponding bone of the castrated male. This actual difference, however, is minimized when it is remembered that the total body length of the normal males is approximately 6 per cent. greater than that of the castrated animal. If we compare the length of the bones as percentages of total body length, we find that each bone of the castrated animal is *relatively* longer than the corresponding bone of the normal animal (length of femur, as percentage of total body length, norm. 15.18 per cent., castrated 15.73 per cent.; tibia, norm. 16.44 per cent., cast. 17.18 per cent.; fibula, norm. 13.8 per cent., cast. 14.3 per cent. Since the relative lengths of the bones are greater in the castrated animals, the conclusion is that castration favors slightly growth in length of hind-leg bones. It must be emphasized, however, that this increase is not marked and careful computation must be employed to make it apparent. It appears so insignificant that little value is attached to it, and certainly the results are not in agreement with those who maintain that castration results in a marked overgrowth in bone lengths.

Spaying.—When the average bone length of the normal female group were compared with those of the spayed female group a decided difference in length was noted; the bone length of the spayed females was greater than similar bone lengths of the normal females. The observed length differences were as follows: femur, 2.4 per cent.; tibia, 3.6 per cent., and fibula 3.9 per cent. longer in spayed females than in normal females. When one considers that the total body length of the normal female was 2.4 per cent. greater than the spayed female, it is apparent that the *relative length* difference is greater than that of the *observed length.* We may conclude from this data that spaying in the guinea pig is conducive to greater lengths in hind-leg bones.

A number of investigators have reported exaggerated bone growth after gonadectomy, particularly in the absence of the testis. Poncet ('03), presenting a résumé of his own earlier experiments, as well as those of Pirsche ('02) and others, maintains that castration is followed by a general increase in the size of the skeleton. Observations on eunuchs, clinical cases of testicular atrophy, and experiments on laboratory animals were cited. In reference to the guinea-pig bones, he found an increase in length of 3 mm. for

the femur and 4 mm. for the tibia in castrated animals; the total lengths were not given, consequently the relative growths of bone in the two cases are unknown. In capons he found an increase of 8 mm. for the femur and 1 cm. for the tibia. Delay in the ossification of the epiphyses following castration is considered the causal factor for increase in length. Sellheim ('99) found delayed ossification in castrated vertebrates (pig, dog, and bull) and a consequent increase in size and length of bones. Hatai ('15) found a very slight increase in the ratio of body length to bone length in castrated rats. He mentions, however, that this difference appears only upon close computation, and is in doubt whether any significance should be attached to his findings.

X. Discussion.

The primary object in conducting the experiment reported herein was to study the reactions in weight of guinea pigs, from birth to maturity, as this may be influenced by the sex glands. Certainly secretions of the sex glands do modify somatic structures in many vertebrates and it has been assumed that weight of laboratory animals such as the rat and the guinea pig reflect, to some degree, their sexual nature; the differences are supposedly detectable if comparisons are made between the weights of normal animals and those having undergone operations at a previous date. However much one may be inclined to doubt the advocacy of utilizing weight as a criterion of sex-gland conditions, an intimate understanding of the reactions are necessary before the doubt can be expressed in definite form.

In the guinea pig (speaking of averages of groups of animals of the same age, same conditions as concerns sex glands, and reared under identical conditions) normal males are constantly heavier than normal females up to the end of the first year; at this time the weights between the two groups are almost identical and, according to Minot, the females subsequently become slightly heavier than the males. But does this mean that the testis promotes, and the ovary retards, growth and increase in weight? Elimination of the gonads of each sex should afford a basis for a partial answer to the question by comparing the growth curves of each group with that of the normal group. Referring to Fig. 1,

it is apparent that the gonadectomized groups fall below the curves of the normal groups for the greater part of the year; but by the 300th day the spayed females have attained the weight of the normal female and remain slightly above these until the close of the experiment. At the end of the experiment, therefore, the spayed females are heavier than normal females, whereas the castrated males are lighter in weight than the normal males. This appears to indicate that the testis does favor growth (absence reduces weight), and that the ovary retards growth (normal females lighter). But let us apply this evidence using as the basis of our comparison four females of the same litter, Y17, Y18, Y19, and Y20. These four sisters, reared together in the same cage, each show gradual growth throughout the year with an entire absence of temporary losses in weight; two had been spayed (Y17, Y18), while the other two remained normal females. On the 360th day each of the two spayed animals weighed 800 grams, one normal female (Y19) weighed 975 grams, and the other (Y20) weighed 745 grams. Since removal of the ovaries causes a relative increase in weight (when averages of entire groups were compared), the spayed females should weigh more than the normal ones. But referring to Table I., it becomes apparent that Y19 (normal) is 22 per cent. heavier than the spayed sisters, whereas Y20 is 2 per cent. lighter than the operated sisters. Our supposition, therefore, is proven that elimination of the ovary leads to relative increase in weight if the comparisons are made between these operated animals and Y20, but it is disproven if the comparisons are made with Y19 representing the normal condition. Consequently, if individual variations in weight are so great with animals of the same litter and lead to such discrepancies in results, one could not expect a more adequate basis of comparison if the animals of unknown history are chosen from the stock at random. One can not fail to be convinced of the magnitude of variation in weight in animals of the same age, sex, and under identical conditions if the weights on the 360th day are compared (Table I.); in some cases the individual variation is greater than 100 per cent. If, therefore, this group of total weights demonstrates nothing more (and indeed little is claimed for it), certainly it shows the fallacy of comparing at random the weights of two, three, or four

animals and claiming for it partial proof for an hypothesis. It appears that the claim of the writer is not only justified, but proven, that the weight of an animal as a character indicating the effect of a sex-gland graft is not only worthless as scientific data, but very confusing to the entire problem of the modifying effects of gonads.

In reference to the effects of gonadectomy on the weight of the glands of internal secretion, little will be added to the discussion in connection with each section of the paper. The great discrepancies in results, even diametrically opposed conclusions, of different investigators working with the same groups of animals have already been pointed out. The chief difficulties in adjusting the results from the various sources appear to center around inadequate controls for the experiments. Should one desire to study the effects of gonadectomy on such a structure as the hypophysis, it should be obvious that animals of similar strains, reared under similar conditions, operated at similar ages, and controlled by the proper groups are essential. Variations in general metabolism are not only so great among animals of different ages and strains that isolated comparisons often indicate inconsistent results, but even animals of the same litter may show marked differences.

The writer does not wish to imply that this experiment has been conducted in an ideal manner, but an attempt has been made to know, in so far as is possible, the history of the animal before and after operation, and to afford comparisons of groups of considerable size rather than mere isolated comparisons. Furthermore, it is realized that unknown conditions may have influenced the results herein reported and repetition is highly desirable before they can be accepted as of particular value.

As to the influence of gonadectomy on bone growth, the above criticisms apply as well. Growth in general body proportions is to a certain extent relatively constant for various parts. It should be supposed at once that a somewhat larger animal than a brother or sister would be expected, in general, to have longer bones and yet not show a specific reaction in bone growth conditioned by the presence or absence of sex-gland secretions.

The results of an analysis of the bone lengths of this group of

animals is surprising from the lack of more appreciable differences. In fact, the most clearly disproportionate length occurred in the spayed females. The bones of this group seem to reflect a specific effect of the absence of the ovarian secretion. The literature leads one to believe that differences between normal male and castrated male bone lengths is pronounced and easily detected. But the difference in lengths among the different groups of this experiment was so slight that careful computation was necessary to reveal it; the difference was so small that it appears almost insignificant.

Striking as may be the influence of the internal secretions of the sex glands on some characters in certain animal forms, it appears difficult and often impossible to discover characters in ordinary laboratory animals that are of sufficient difference and constancy in the two sexes to be capable of analysis by experimental procedure. And many of the characters cited in the literature supposedly offering a demonstration of the power of sexual secretions to effect modifications in the opposite sex fall to the ground if subjected to critical analysis. In the writer's opinion the character of weight reactions in guinea pigs belong to this group.

XI. Summary and Conclusions.

On the basis of observations on the guinea pig, herein reported, the following conclusions are drawn:

1. The curve of growth for normal male guinea pigs is consistently above that for normal females up to the end of the first year, when the two curves practically coincide.

2. Gonadectomy is followed by a decrease in the growth curves for both sexes; however, by the 300th day spayed females have reached the weight of normal females, and at the end of one year are 1 per cent. heavier than the normals.

3. The relative weight of a guinea pig is worthless as an indication of its sexual condition; properly controlled groups of weights may offer a debatable criterion of sex-gland effects, but random comparisons indicate nothing.

4. Total length of the animals correspond to total weights in the order: normal males > normal females > spayed females > castrated males.

5. The relative weight of the hypophysis of normal females is slightly greater than that of the normal males at the end of the first year.

6. Gonadectomy is followed by a relative reduction in weight of the hypophysis in operated males and females.

7. Thyroids of normal females are slightly heavier than those of normal males.

8. Gonadectomy appears to favor growth of the thyroids in both operated males and females.

9. Adrenals of normal male guinea pigs are approximately 20 per cent. greater in weight than those of normal females.

10. Gonadectomy is followed by a relative decrease in weight of adrenals in both sexes; though slight differences appear in males, the effect is considerably more pronounced in females.

11. The normal female spleen was found to be slightly heavier than that of the normal male.

12. Gonadectomy appears to cause a reduced growth of the spleen in both sexes.

13. The length of the hind-leg bones of the normal male is slightly greater than that of the normal female.

14. Castration favors, to a slight extent, the growth of leg bones in operated males, though the differences appear to be relatively insignificant.

15. Spaying is conducive to bone growth in operated females; the relative differences are considerably more pronounced than those following castration.

16. Repetition of the experiment is deemed highly advisable before the results, herein reported, are accepted as indicative of general principles.

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